INFLUENCE OF DIET ON PERFORMANCE PARAMETERS, INTESTINAL LESION DEVELOPMENT, AND OOCYST CYCLING IN LIVE OOCYST VACCINATED REPLACEMENT BROILER BREEDERS

A Thesis

by

LESLEE ANN ODEN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2009

Major Subject: Poultry Science

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Approved by:

Chair of Committee,	Jason Lee
Committee Members,	David Caldwell
	Glenn Holub
Head of Department,	John Carey

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ABSTRACT

Influence of Diet on Performance Parameters, Intestinal Lesion Development, and Oocyst Cycling in Live Oocyst Vaccinated Replacement Broiler Breeders.

(August 2009)

Leslee Ann Oden, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Jason Lee

Two consecutive experiments were conducted to evaluate the influence of dietary composition, specifically protein and amino acid profile, on performance parameters, oocyst output, and lesion development in male and female replacement broiler breeders of two different genetic lines vaccinated with a live coccidiosis vaccine. Dietary formulations were based on either breeder specific recommendations or formulations of a broiler integrator. On day 28, males of each genetic line were added to female pens to evaluate the effect of co-mingling on male performance. Lesion assessment was performed on three separate occasions per each experiment. Fecal material was collected to determine oocyst cycling patterns.

During experiment 1, flock uniformity was improved ($P \leq 0.05$) in Line A males fed the integrator diet. Increased body weight and improved uniformity of Line B females was observed with the breeder recommended diet. Co-mingling negatively impacted ($P \leq 0.05$) male body weight. Multiple oocyst peaks were observed in both genetic lines, with the first peak occurring at approximately 16 to 18 days post

placement. This first peak tended to have the highest observed magnitude and corresponded with the highest level of intestinal lesions observed during the experiment.

In experiment 2, diet impacted ($P \leq 0.05$) average body weight in Line A males, Line B males, and Line B females. Line A males fed the breeder recommended diet had increased ($P \leq 0.05$) body weight at the termination of the experiment. Line B males and females fed the breeder recommended diet had increased ($P \leq 0.05$) body weights throughout the experiment beginning on day 7. Negative effects ($P \leq 0.05$) on male body weight resulting from co-mingling were observed. Oocyst peaks were delayed and at a lower magnitude in both genetic lines compared to peaks observed in experiment 1. Dietary interactions were observed in both experiments where magnitude of peak, duration of oocyst output, and severity of lesion development was influenced by diet in both male and female genetic lines. These data indicate that co-mingling negatively impacts male performance and dietary composition can impact male and female performance, oocyst cycling, and lesion development during coccidiosis vaccination in replacement broiler breeders and should be taken into consideration when rearing replacement broiler breeders.

DEDICATION

I would like to dedicate this thesis to my parents, siblings, aunt and uncle, and grandmother. Your love and support has given me the motivation to accomplish my dreams. Mom and Dad, you both complete the definition of amazing parents. You have raised me to be a strong and hard working young woman and I am truly blessed to have such loving parents in my life. To my brother, sister, aunt, uncle and MeMaw, thank you for constantly showing me the power of family and love, you all have helped me conquer my hopes, fears, and dreams. I love you all from the bottom of my heart and I could not have accomplished this without your help. I would also like to dedicate my work to my late grandparents. Even though they only live within me through spirit, I am forever guided in life by them every day. They are my guardian angels and are watching over me helping me tackle life through indivisible love. I love you and thank you for being my wonderful family.

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CHAPTER I

INTRODUCTION

Coccidiosis is a disease of poultry that is caused by a single celled protozoan parasite of the genus *Eimeria.* *Eimeria* are intracellular parasites which invade the intestinal mucosa resulting in the induction of epithelial cell damage and intestinal inflammation. Infections caused by coccidian parasites have been shown to have a major economic impact on the United States poultry industry (Yun et al., 2000). The nature and life cycle of the organism render it nearly ubiquitous in the live poultry industry and it is therefore virtually impossible to completely eradicate the problem. Avian *Eimeria* spp. replicate by a complex life cycle comprised of three different phases which include: sporogony, merogony, and gametogony (Allen et al., 2002). The infective unit of this complex cycle is an 'oocyst'. Oocysts are shed in the feces of the bird and sporulate in the litter of the poultry house. Sporulated oocysts are the infective stage for live poultry and once ingested, they are able to promote infection and proliferation. Exposure to live oocysts is necessary to obtain complete or partial immunity against the effects of this organism.

Broiler breeders, whose progeny are the birds which are raised for commercial broiler production, are not isolated to the threat of the coccidian parasite and thus control measures must be put into place in order to prevent outbreaks within the breeder flocks. It is essential for breeders to develop immunity to diseases. Anticoccidials are

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This thesis follows the style of Poultry Science.

efficacious at preventing the disease, but a major drawback of the use of anticoccidial drugs is the development of resistance (Peek and Landman, 2006). However, there are few anticoccidials that are approved for use in breeders, therefore, it is essential for birds to develop immunity through the use of vaccines (Leeson and Summers, 2000). Vaccines are used to accelerate the bird's immune system by initiating an immune response, without causing actual disease. The overall aim of a vaccination program in breeders is to protect the juvenile and adult broiler breeders from infection while ensuring optimum maternal antibody transfer to offspring (Leeson et al., 2000). Various methods of approach are taken in an effort to achieve this objective with the most popular of those alternatives being the administration of live oocyst vaccines on day of hatch. The administration of vaccine, and subsequent immunity, in conjunction with factors including diet, rearing conditions, age, and immune status are all responsible for the development and overall performance of the animal.

An important part of breeder nutrition is to maintain dietary specifications and feed intake. Providing well balanced diets is critical for a successful feed management program (Leeson and Summers, 2000). The most critical components of broiler breeder diets are energy, calcium, and protein or amino acids. Dietary recommended specifications can vary according to strain of the primary breeder. During the early rearing period, adequate frame size and body weight are developed. Feeding a diet with higher crude protein during the starter period generally stimulates early body weight gain and skeletal growth (Hudson et al., 2000). As the bird ages, additional protein serves to maintain flock uniformity and to ensure a proper degree of fleshing on the bird (Joesph et al., 2000). There are few reports of

interactions between the effects of dietary protein intake and live oocyst vaccination as they influence early performance of broiler breeders.

Therefore, the two current experiments were designed to investigate the effect of dietary composition on male and female replacement broiler breeder performance, oocysts cycling, and lesion development in two commercially available genetic lines which were vaccinated with a live oocyst vaccine on day of hatch. In addition, the effects of the co-mingling of replacement broiler breeder cockerels with pullets was investigated to determine whether measurable effects were rendered on male performance. This data will provide the broiler breeder industry information about the interactions of performance and diet when utilizing live oocysts vaccines as the preferred coccidiosis control measure.

CHAPTER II

REVIEW OF LITERATURE

Eimeria LIFE CYCLE

Coccidiosis is a disease of poultry that is caused by a single celled protozoan parasite of the genus *Eimeria. Eimeria* are intracellular parasites that invade the intestinal mucosa and induce epithelial cell damage and inflammation. Infections caused by coccidian parasites have had a major economic impact on the U.S. poultry industry (Yun et al., 2000). Surveys conducted by McDougald and colleagues, (1986) revealed that the presence of coccidiosis is ubiquitous among most broiler farms. The omnipresent nature of poultry coccidia precludes the possibility of elimination or prevention of coccidia by quarantine, disinfection, or sanitation (Calnek, 1997). Coccidial infections are self-limiting, depending largely upon the number of oocysts ingested and on the immune status of the host organism. Species of coccidia are identified on the basis of oocyst morphology, host specificity, immune specificity, appearance, location of gross lesions within the natural host, and length of the prepatent period (Calnek, 1997). The host specificity of *Eimeria* in birds and mammals is very strict, in that parasites from different species of birds or animals can be considered different species even though they may have similar appearing oocysts. There are eight species of chicken coccidia which include *E. acervulina, E. brunetti, E. maxima, E. mivati, E. mitis, E. necatrix, E. praecox,* and *E. tenella* (Conway et al., 2007). Each of these species develops in a particular location within the digestive tract, each having its own

characteristics in respect to prevalence, site of infection, pathogenicity, and immunogenicity (Vermeulen et al., 2001).

Avian *Eimeria* spp. have a life cycle comprised of three different phases: sporogony, merogony, and gametogony (Allen et al., 2002). Oocysts, which are shed in feces, undergo sporogony in the external environment. After being in the litter for 24 hours, oocysts sporulate and become infective to the chicken. The sporulated oocyst contains four cysts known as sporocysts with each sporocyst having 2 infective parasites known as sporozoites (Fetterer et al., 2003). Once ingested, the sporozoites are released into the intestine and attach to the intestinal cell lining. Within the host cells, the asexual phase of the life cycle (merogony) occurs with the multiplication of the parasite in the internal area of the intestine (Allen et al., 2002). The multiplication results in a large number of daughter parasites which are contained in a large body known as a schizont. When the schizont fills up with parasites, it ruptures and releases the daughter parasites into the internal side of the intestine resulting in the destruction of the intestinal cell lining (Allen et al., 2002). This impairs the intestine's ability to absorb nutrients due to the changes in the permeability of the gut wall. This also alters the normal passage of proteins and fluids across the intestinal barrier, allowing for wetter droppings which favor the growth of pathogenic bacteria such as *Clostridium perfringens* (Allen et al., 2002). Sexual reproduction or gametogony follows the last merogonic cycle. Merozoites enter host cells and develop into either male (microgamonts) or female (macrogamonts) forms. The microgamonts give rise to many microgametes, which exit, seek, and penetrate the macrogamonts resulting in more destruction of the intestinal lining and the production of immature oocysts (Allen et al., 2002). The oocysts

are then passed through the droppings onto to the litter to complete the life cycle. The development of immunity depends on the repeated completion of coccidial life cycles as chickens re-infect themselves by foraging in the litter (Williams, 2002).

The species of *Eimeria* in the fowl have been studied in great detail (Vermeulen et al., 2001; Allen et al., 2002; Chapman, 2009). Many of the features of disease in the host are expressions of the characteristics of the individual species of *Eimeria,* and as such, are often used as a guide to identification. Most species are pathogenic to varying degrees, and can be identified by using the characteristics of the lesions that develop, their location, and association with developmental stages of the parasites (Joyner, 1982). It is common to find at least six species (*Eimeria acervulina, E. maxima, E. tenella, E. brunetti, E. mitis,* and *E. praecox*) in litter samples from a single flock during a six week time frame (Allen et al., 2002). Five of the species *E. acervulina, E. brunetti, E. maxima, E. necatrix, and E. tenella* are well known and identifiable with relative ease, because they produce characteristic gross lesions with pathogenicity ranging from moderate to severe. Interactions between species can result in an exacerbation of total effects or modifications in activity of individual species. Hein (1976) investigated the pathogenicity of *E. acervulina, E. brunetti*, and *E. maxima*. Generally, weight loss was shown to increase in cases enduring infection with multiple species, but competition showed a tendency to reduce oocyst production attributable to one given species in the presence of another.

In the chicken, species of *Eimeria* parasitize and develop in different regions of the gut with *E. acervulina* occupying the most proximal region and *E. tenella* and *E. brunetti* in the most distal regions. In addition, different stages of a single species can be specific to

different regions of the intestine and to different cell types within that region. *Eimeria acervulina* commonly invades the duodenal loop of the intestine, and in severe infections can progress into the proximal to mid jejunum. *E. acervulina* attacks primarily older chickens and replacement hens (Conway and McKenzie, 2007). This species has a low reproductive potential and the immunity that develops after infection is weak (Pellérdy, 1974). Mortality is low, but since the infection disrupts normal digestive functions the affected chickens or hens lose weight and egg production falls (Pellérdy, 1974). *Eimeria mivati* is frequently diagnosed as *E. acervulina* because of the site of infection. *E. mivati* also parasitizes the upper small intestine, however, can infect sections of the lower intestine as well (Conway and McKenzie, 2007). *Eimeria maxima* infections are located in the mid-intestinal region (either side of meckels diverticulum, also known as the jejunum and ileum) of the bird. It has been named for its large oocyst, and occurs mostly in older chickens and laying hens (Pellérdy, 1974). *Eimeria brunetti* parasitizes the lower intestine, extending into the large intestine. Early stages of infection frequently invade the mid-intestinal area (Conway and McKenzie, 2007). The two most highly pathogenic species are *Eimeria tenella* and *E. necatrix*. *Eimeria necatrix* lesions are also associated with the mid-intestinal area; however, oocyst development occurs later in the ceca of the bird. Infections with *E. necatrix* occur most frequently in chickens aged 5 – 7 weeks (Conway and McKenzie, 2007). *Eimeria tenella* invades the two ceca and in severe cases may also parasitize the intestine above and below the cecal junction (Conway and McKenzie, 2007). *E. tenella* generally affects young chickens, and in severe infections causing mortality at 5 – 6 days of age (Pellérdy, 1974). Older chickens and hens only rarely develop *E. tenella* infections,

although it is not because of resistance conferred by their age, but because of repeated small infections during their life which enables an effective active immunity to build up (Pellérdy, 1974).

Many factors have been shown to influence the severity of *Eimeria* infection and thus the outcome of the disease, such as those factors which are inherent within the particular isolate or strain, factors associated with the inside environment of the host and the outside environment acting on the parasite, as well as factors associated with the genetic makeup and immune status of the host (Joyner, 1982). The reaction of the host to coccidial infections depends on the number of sporulated oocysts ingested (Joyner, 1982). An increase in the number of oocysts ingested by the host is usually accompanied by an increase in the severity of the disease (Long 1973). The exposure of the host to massive numbers of *Eimeria* in the early parasitic stage may stimulate immune mechanisms which can be effective against later stages in the life cycle. The viability of oocysts and their ability to infect a host are affected by many environmental factors. Sporulation of the oocyst depends mainly on three basic factors; temperature, humidity, and access to oxygen (Kheysin, 1972). Under ideal conditions, sporulation occurs in 24 to 48 h for most poultry *eimerian* species (Edgar, 1955). Oocysts must undergo sporulation before they are infective; this process is dependent on temperature, humidity, and oxygen tension. In addition to molecular changes during sporulation, it has been well documented that mannitol, which is in high concentration in the oocyst, is the primary energy source for sporulation (Schmatz et al., 1977; Milkaski et al., 1994; Allco et al., 1999).

The infective form of *Eimeria* is the highly resistant oocyst, which is shed in the feces of infected animals. The oocyst is excreted from the host as an undifferentiated stage, and in order to become infective it must sporulate. The degree and rate of sporulation of excreted oocysts are important factors affecting the infection pressure in a flock of birds, thus influencing the epidemiology of the infections. It is generally believed that moist litter will favor the development of coccidiosis, because of the higher sporulation ability of such an environment of such an environment (Card and Nesheim, 1972). Graat et al. (1994) studied the sporulation of oocysts of *Eimeria acervulina* in dry and moist litter with different combinations of temperature and relative humidity and found only a slight difference in sporulation rates with changes in temperature and relative humidity. The maximum proportion of oocysts of *E. acervulina* that sporulated did not vary with temperature or relative humidity. Waldenstedt et al. (2001) studied the degree of sporulation of *E. maxima* oocysts at different moisture levels in the litter at a fixed temperature and relative humidity. Sporulation of *E. maxima* was most efficient under the driest conditions studied (16% moisture content), and poorest in the samples with the highest moisture content (62%). Even though the differences may not have resulted from a direct effect of humidity on the oocysts, but more likely resulted from limited oxygen in the more moist substrates. This demonstrates that sporulation is not favored by moist litter. *Eimeria* species have different multiplication capacities and different sensitivities to competition with other species of coccidia or other infectious agents (Brackett and Bliznick, 1952; Williams, 1973), which might influence the impact of the course of sporulation during an *Eimeria* infection. Oocysts

of different *Eimeria* species may also differ in their ability to withstand harsh environmental conditions.

Localized intestinal parasitic infections have been shown to profoundly affect the degrees to which certain nutrients can be absorbed from parasitized intestines. The amount of a nutrient absorbed from a parasitized intestine was dependent upon the nutrient examined, the area of the intestine affected by the parasites, and the stage of the parasitic infection. A study conducted by Turk (1972) observed the effects of intestinal parasitism upon the digestion and absorption of the amino acids from proteins. The results showed that duodenal infections with *E. acervulina* resulted in minor alterations in protein digestion and absorption. Infections of the ileum, cecum, and colon with *E. brunetti* resulted in very small changes in protein digestion and absorption. These results suggest that these areas of the intestine play little or no role in protein digestion and absorption.

LIVE OOCYST VACCINATION

Coccidiosis is an on-going problem in breeders and it is important to have preventive programs in place. It is essential for breeders to develop immunity to diseases. Anticoccidials are efficacious at preventing the disease, however they inhibit the bird's ability to develop immunity in the process, and thus a major drawback of the use of anticoccidial drugs is the development of resistance (Peek and Landman, 2006). Over time, the proportion of coccidia that are resistant to the drugs increases and the result is the development of more severe lesions, which can lead to the development of poor uniformity and adversely affect frame size (Leeson et al., 2000). To minimize the occurrence of anticoccidial drug resistance, a rotation of various anticoccidial drugs, otherwise known as a

shuttle program, is used (Peek and Landman, 2006). However, there are few anticoccidials that are approved for use in breeders, therefore, it is essential for birds to develop immunity by use of other methods, which can be accomplished with the use of vaccines (Leeson et al., 2000). Vaccines are used to accelerate the bird's immune system by initiating an immune response to antigens present within the vaccine, but without causing actual disease. The overall aim of a vaccination program in breeders is to protect the juvenile and adult broiler breeders from infection while ensuring optimum maternal antibody production in the offspring (Leeson et al., 2000).

Live oocyst vaccines are comprised of several species of *Eimeria* that can be administered in several ways to chicks. The efficient induction of protective immunity using live vaccines is as equally crucial as the even distribution of the vaccine oocysts over the birds (Vermeulen et al., 2001). Methods of administration of live oocyst vaccines include feed application, water application, and gel administration, although the most commonly used method in commercial industry is the use of spray cabinets (Williams, 2002). This method consists of spraying the vaccine over trays of hatched chicks and allowing the chicks to preen, thus encouraging the ingestion of the live oocysts in the vaccine. The hatchery spray method of vaccination has been largely responsible for increased use of anticoccidial vaccines in the broiler market (Williams, 2002). It is essential to have uniform exposure to all chicks, because any non-vaccinated birds will be naturally infected in five to seven days post vaccination as the vaccinated chicks begin to shed oocysts onto the litter (Leeson et al., 2000). Natural cycling is important for vaccinated chicks because it takes two cycles of *Eimeria* before complete immunity is established (Calnek, 1997). When vaccination is used

to control coccidiosis, the risk of contracting coccidiosis is highest at an early age (weeks 1-3) and decreases as immunity is developed from weeks 3 to 4 onwards (Vermeulen et al., 2001). A study by Williams and colleagues (2000) observed oocyst production and cycling patterns on new and recycled litter in order to establish the duration of immunity that is maintained in broiler breeders. Results showed that after a single administration of a live oocyst vaccine, low levels of oocyst shedding began to appear at seven days of age. The low level peaks were followed by a higher incidence of shedding between 35 and 42 days of age following vaccination. The long-term persistence of oocysts in the litter of partially immune birds results from multiple re-infections by local heterologous coccidial populations (Williams et al., 2000). Hence, the continuing low level of oocyst production by the partial immune birds attains equilibrium with the destruction of oocysts in the litter.

Beach and Corl (1925) first noted that chickens infected with live coccidia became resistant to challenge with the same parasite. Hein (1976) later showed that growth performance was not affected and oocyst production was negligible after re-infection of chickens immunized with live oocysts of *E. acervulina*. This study also discovered that the dose of oocysts was critical for the development of full immunity. Only partial resistance to reinfection was achieved by immunization with two doses of *E. acervulina*. However, when three low doses of oocysts were used in an effort to induce resistance, long-lasting immunity was achieved. This was confirmed with the observation of resistance to reinfection when high-dose challenges of *E. acervulina, E. brunetti* or *E. necatrix* were administered. Pathological effects of the live coccidial oocysts prevented higher doses from being tested. It is also important to maintain a minimum of 14 days between primary and secondary

infections to avoid interference during the second infection due to tissue damage caused by the initial infection.

Several live coccidial vaccines are available commercially. The Immucox® vaccine (Vetech Laboratories, Buffalo, NY) is composed of infective oocysts from different species of *Eimeria* delivered in drinking water. Danforth *et al.* (1989) compared four methods of delivery of Immucox® (gavage, spray-cabinet, feed and gel delivery), and found gel delivery to be superior to the others. Shirley and Bedrnik (1997) selected precocious strains of *E. acervulina* and *E. tenella*, which are included as oocysts together with an egg-adapted line of *E. tenella* in a commercial live vaccine (Livacox®, Biopharm, Prague, Czech Republic). The Immucox® (Vetech Laboratories, Buffalo, NY), Livacox® (Biopharm, Czech Republic), and Coccivac® (Intervet Schering-Plough, Summit NJ) vaccines may not contain sufficient numbers of the more pathogenic species to induce long-lasting protective immunity, and consequently their efficacies depend on auto re-infection from recycled parasites (Lillehoj et al., 2000). Since pathogenicity occasionally predominates over immunogenicity, live vaccines may introduce new species or unexpected pathogens into a flock (Lillehoj et al., 2000). Several methods of modulating the pathogenicity of live *Eimeria* vaccines have been attempted. These include giving multiple low-dose inoculations over a long period of time to young birds (trickle immunization), *in ovo* inoculation and the co-administration of anticoccidial drugs with vaccines. The trickle immunization method produces stronger and longer-lasting immunity than a single immunization containing the same number of oocysts (Lillehoj et al., 2000). Chickens immunized with live *E. acervulina*, either by the trickle procedure or in a single dose, both demonstrated resistance to re-infection, as evidenced by

reduced fecal oocyst output, and reversal of growth reduction compared with non-immunized controls (Galmes *et al.*, 1991). Prolonged exposure of chickens to *E. tenella* was shown to induce protective immunity against challenge by the homologous parasite (Nakai *et al*., 1992).

The ability of *Eimeria* given repeatedly to protect against heterologous challenge was investigated using a foreign host (Augustine *et al.*, 1991). These studies demonstrated only partial success in chickens inoculated recurrently with oocysts of the turkey coccidium, *E. adenoides,* and challenged with the chicken coccidium *E. tenella*. Watkins *et al.* (1995) speculated that parasites introduced *in ovo* might complete their life cycle within developing chicks and thereby induce protective immunity; however, the investigators were unable to demonstrate such protection after *in ovo* administration of either *E. maxima* oocysts or sporozoites. Inoculation of adult chickens with viable *Eimeria* parasites in the presence of drugs that inhibit parasite development (Long and Jeffers, 1982) has also produced inconsistent results. Live vaccines allow for a gradual build up of solid immunity as a result of recycling of the parasites through several life cycles over a period of about 4 weeks. The uses of such vaccines have been successful in broiler breeders.

BROILER BREEDER MANAGEMENT

The overall objective of the broiler breeder industry is to successfully produce broiler breeder pullets and cockerels with the potential to maximize the production of saleable chicks (Griffin et al., 2005). There are many factors that are associated with successful management of replacement broiler breeders including the control of average body weight, uniformity, feeding program, appropriate lighting schedule, environmental management, and

disease control. The early rearing period is a critical period for establishing adequate frame and body weight (Hudson et al., 2001). When birds are grown improperly during early rearing stages, reproductive efficiency of the flock is negatively affected.

Several published reports have been conducted to determine effective nutritional methods for controlling body weight at different growing phases to allow for improved adult performance of broiler breeders (Lopez et al., 1994). The growth of females and males is governed by the feed allowance and feeding program. Body weight targets in the early rearing stage (0 – 4 weeks) are achieved by *ad libitum* feeding or full feed access. This is to ensure that chicks are developing a healthy appetite and achieve optimum growth. Once the birds have reached target weight it is important to maintain body weight and flock uniformity, daily feed allowance and skip-a-day feeding programs have been used successfully in controlling body weight (Lopez et al., 1994). The basic concept of skip-a-day feeding, where birds are fed on an every other day basis is to ensure the quantity of feed is evenly distributed so that smaller, more timid birds can still eat and maintain proper weight and uniformity (Leeson and Summers, 2000). When using the skip-a-day feeding program, birds must store nutrients (body fat and body protein) which will be utilized for growth and maintenance when there is no access to feed. Uniform flocks with appropriate weight during early rearing have several advantages in future performance.

DIETARY INFLUENCE

An important part of breeder nutrition is to maintain dietary specifications and feed intake. Providing well balanced diets is critical for a successful feed management program (Leeson and Summers, 2000). The most critical nutrients for broiler breeders are energy,

calcium, and protein or amino acids. Dietary recommended specifications can vary according to strain of the primary breeder. During the early rearing period, adequate frame size and body weight are developed. Feeding a diet with higher crude protein during the starter period generally stimulates early body weight gain and skeletal growth (Hudson et al., 2000). As the bird ages, additional protein serves to maintain flock uniformity and to ensure a proper degree of fleshing on the bird (Joesph et al., 2000). There are few reports of interactions between the effects of dietary protein and energy intake where early performance of broiler breeders is influenced.

A study conducted by Lilburn et al, (1987) evaluated the effects of protein levels at various stages of development. Broiler breeder pullets were fed rations containing 13.5 and 15.5 % crude protein from 0 to 5 weeks of age. During this time period body weight gain was monitored to observe differences between the two diets. Pullets fed the 13.5 % protein diet yielded significantly increased body weight gain compared to the pullets fed the 15.5 % protein diet. Lilburn et al, (1986) reported that a high protein starter treatment was not beneficial to the young pullets because the physiological parameters studied may have some negative interactions with the onset of feed restriction. The experiment continued through the beginning of egg production to evaluate overall dietary effects. The study showed that protein has less influence on body weights but may significantly influence subsequent breeder performance. The production response to dietary protein fed during the pre-breeder phase may be due to several factors. During this time, important aspects of reproductive development are taking place (Schjeide et al. 1963; Yu and Marquardt, 1974). To achieve the necessary growth and development required for maximal production more refined

dietary needs during this critical period need to be considered. Body weight alone has been the best management tool available for growing breeder pullets. Reports suggest that changes in diet may significantly affect production without influencing body weight gain (Liburn et al, 1987; Cave, 1984; Brake et al, 1985). Protein has been the nutrient studied to the greatest extent but the significant interaction between energy and protein reported by Brake et al. (1985) suggests that other dietary factors are important. For broiler breeders, at 20 weeks of age placed on litter, protein intake of 20.6g/bird per has been recommended (Wilson and Harms, 1984). At feed intake of 150 g/ bird per day, the diet would contain 13.7 % crude protein, which is considerably less than breeder guidelines suggest. Waldroup et al. (1976) found that the protein requirement over the entire production period of broiler breeders received a corn-soybean meal diet without supplemental amino acids were approximately 20 to 22 g per day. For breeders in floor pens, 19.5 g per day was adequate when amino acid profile was maintained (Pearson and Herron, 1981), and 16.5 g/d was adequate for individually caged breeders (Pearson and Herron, 1982a). Proudfoot (1980) determined that a 13.6 % protein breeder diet was adequate to support optimum performance. Excessive amounts of crude protein have been proven to be unnecessary for optimum performance of broiler breeder hens and have also been associated with negative effects on future reproductive performance (Lopez et al., 1995).

For broiler breeders, feed restriction is necessary to control body weight, because it can improve egg weight and egg production (Siegel and Dunnington, 1985). Unfortunately, an appropriate level of crude protein for broiler breeders has not been determined. Several studies have shown that protein levels of 10 to 14% CP satisfy the protein requirement of

hens already in lay (Waldroup et al., 1976; Bornstein et al., 1979; Lopez and Leeson, 1995). Bowmaker and Gous (1989) calculated that a broiler breeder pullet would require approximately 10 g of crude protein per day before the onset of lay, in addition to the amount needed to sustain egg production. Breeder companies often suggest having as high as 15 to 16% crude protein in the breeder diet. As the bird ages, additional protein serves to maintain flock uniformity and to ensure a proper degree of fleshing on the bird. A high level of dietary protein also maximizes the amount of body protein available for egg formation and egg production (Joseph et al., 2000).

Body weight is still the only practical tool for monitoring the progress of a given restriction program, but it is recognized that different planes of nutrition can influence body composition and skeletal development in pullets with similar body weight (Lilburn *et al*., 1989; Lilburn and Myers-Miller, 1990). In a study by Yassile and Lilburn (1998), the primary goal of the experiment was to study the relationship between protein intake, body weight gain, and breast muscle weight in pullets fed diets of similar energy (ME) content from day 0 to 5 weeks of age. All pullets were fed a 19% crude protein starter diet from 0 to 6 day of age and every other day feed restriction was implemented on day 7. Beginning with the onset of restricted feeding, pullets were fed either a 15% crude protein diet, 17% crude protein diet, or 19% crude protein diet. The different levels of dietary protein after 7 days of age did not significantly affect body weight at day 14 or 28. These effects during the early growing period certainly support the concept that with no changes in target body weight, protein intake could have independent effects on reproductive performance of breeder hens as previously reported (Cave, 1984; Brake *et al*., 1985; Lilburn *et al*., 1989;

Lilburn and Myers Miller, 1990). It remains to be seen, however, whether these effects of protein are specific to the pre-breeder period, as reported in the previous citations, or are a function of cumulative protein intake over the entire growing period, as suggested by Walsh and Brake (1997).

Dietary protein retention has been reported to decrease during a coccidial challenge (Sharma and Fernando, 1975) and other studies have reported that feeding high levels of dietary protein enables birds to better cope with a coccidial challenge (Harms *et al.*, 1967; Welch *et al.*, 1986). Yassile and colleagues (1999) evaluated the interaction between dietary protein intake and the response to a coccidial challenge by feed restricted broiler breeder pullets from 0 to 6 weeks of age. The experiment included an arrangement of treatments with 2 levels of dietary protein and 3 levels of vaccine dose. The dietary protein levels evaluated were 15% and 19% to observe any differences among performance parameters. The results of the study showed that there were no significant effects of vaccine dose on average pen body weight, but pullets fed the 19% crude protein diet were significantly heavier at 14, 28, and 35 day of age. Throughout the course of the study, there was a consistent increase in body weight of the pullets fed the 19% crude protein diet. With respect to intestinal development or resistance to a mild coccidial challenge, there appears to be no significant benefit to increasing dietary protein levels in restrict-fed pullets. These results demonstrate that increased protein intake can have a positive effect on body weight during feed restriction and a coccidial challenge.

In conclusion, performance of broiler breeders has been shown to be affected by dietary composition. Varying protein levels and vaccination influence the potential outcome

of the breeders' performance. With these findings, we hypothesize that dietary composition and male co-mingling will influence performance parameters in vaccinated replacement broiler breeders. Two experiments were conducted to evaluate the effect of dietary composition on performance parameters, male co-mingling, intestinal lesion development, and oocyst output in male and female replacement broiler breeders of two genetic lines following live oocyst vaccination.

CHAPTER III

INFLUENCE OF DIET ON PERFORMANCE PARAMETERS IN LIVE OOCYST VACCINATED REPLACEMENT BROILER BREEDERS

INTRODUCTION

Avian coccidiosis is responsible for substantial economic losses in the poultry industry world-wide (Allen and Fetterer, 2002; Morris and Gasser, 2006; Shirley et al., 2007). Coccidiosis is caused by *Eimeria* which are intracellular protozoan parasites that invade the intestinal mucosa and induce epithelial cell damage and inflammation (Adams et al., 1996; Duffy et al., 2005). Coccidiosis is a constant problem in the US poultry industry requiring producers to have preventive programs in place. There are few anticoccidials approved for use in breeders, it is therefore essential for birds to develop immunity to these organisms. Vaccination is an effective means to prevent and/or reduce the adverse effects of specific diseases in poultry (Danforth et al., 2002; Peek and Landman, 2006; Mathis and Broussard, 2006). Immunity to coccidiosis can be developed with the use of vaccines containing live oocysts derived from several *Eimeria* species (Vermuelen et al., 2001). In the U.S., administration of live vaccines is a common practice used in broiler breeder rearing and production (Chapman et al., 2002).

The early rearing period is critical for establishing adequate frame size and body weight (Hudson et al., 2000), and control of immature body weight is critical for future performance and productivity of a broiler breeder flock (Harms, R.H., 1992; Lopez and Leeson, 1994). Dietary composition influences production parameters during avian clinical

coccidiosis (Garcia Neto et al., 2000) and vaccination. Previous research has demonstrated increased broiler breeder pullet body weight as a result of increased protein levels (15% vs. 19%) during coccidiosis vaccination (Yassile et al, 1999). However, this range of 4% between the two levels may not accurately represent protein levels in replacement broiler breeder diets that are currently being utilized in replacement broiler breeder rearing. Management guides provided by primary breeders include dietary recommendations, however, in the interest of cost savings and convenience, these recommendations are not always followed. Management guides also recommend rearing male and female replacement broiler breeders separately, however, the practice of rearing co-mingled replacement broiler breeders in the commercial poultry industry is common. Therefore, the current study was conducted to determine the effect of co-mingling on male performance and to evaluate early male and female replacement broiler breeder performance following coccidiosis vaccination when fed a diet which meets recommendations of the primary breeder or a diet that has been commonly fed by a commercial broiler integrator.

MATERIALS AND METHODS

This study consisted of two consecutive experiments following the same experimental design. The first experiment was conducted on fresh pine shaving litter material and the second conducted on built up litter following the first experiment. Animal care was provided in accordance with a protocol approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC).

Animals and Management Practices

A total of 480 broiler breeder males (240 Line A and 240 Line B) and 1152 broiler breeder females from two different commercially available genetic lines (576 Line A and 576 Line B) were placed in floor pens and fed either the integrator diet or genetic line specific breeder recommended diet. The males were housed in six replicate pens per group resulting in 24 total pens (12 per genetic line) at a final placement density of 1.75 ft^2 per bird. Females were housed in eight replicate pens per group resulting in 32 total pens (16 per genetic line) at a final placement density of 1.5 ft^2 per bird. Females and males were given access to half of the pen through days 14 and 28, respectively, then full pen access was given for the remainder of the experiment. All chicks were vaccinated with a live oocyst vaccine (Coccivac®-D[1]) at the hatchery of the primary breeder prior to delivery. Upon arrival, males and females of each genetic line were wing-banded, weighed, and randomly assigned to treatment based on placement weight.

Both males and females of each genetic line were subjected to the following lighting schedule in a dark out rearing house: 24 hours of light with an intensity of 30 lux (Day 0-3), 18 hours of light with an intensity of 30 lux (Day 4-7), 12 hours of light with an intensity of 15 lux (Day 8-21), 8 hours of light with an intensity of 15 lux (Day 22-42).

Dietary Program

Each genetic line was fed one of two diets (breeder recommended diet or the integrator diet). The integrator diet was the same for both genetic lines and there was not a difference in dietary specifications based on sex. The only significant difference between

[1] Intervet Schering-Plough Animal Health, Summit, NJ

the two breeder recommended diets and the integrator dietary program was protein concentration and amino acid profile, with the integrator having a lower concentration of protein. Feeding programs and schedules were specific with regard to genetic line. All Line A breeders were switched from a starter to grower diet on day 21 which follows the recommendation of the primary breeder and integrator programs. Breeder recommendations for Line B required that a starter diet be fed through six weeks of age; however, Line B breeders fed the integrator program were switched from a starter to grower diet on day 26 to maintain the integrator management program. Feed restriction for both genetic lines began on day 21 (Experiment 1) or day 14 (Experiment 2) with daily allocations and skip-a-day feeding commencing on day 28 and persisting through the completion of the experiment on day 42. All replacement breeders were individually weighed weekly through day 42, to determine average body weight and pen uniformity.

Co-mingling vs. Separate Rearing of Males

To evaluate the effect of co-mingling on male performance, males and females were reared separately until four weeks of age. On day 28, one replicate pen of males per treatment was distributed into half of the female pens of identical treatment. The remaining five replicate pens of males continued to be reared separately until the conclusion of the study. Feed allocations for co-mingled replicates were adjusted to maintain recommended daily intake.

Statistical Analysis

Average body weights, pen coefficient of variation for males and females of each genetic line were analyzed via a one-way Analysis of Variance (ANOVA) using the General

Linear Model. Differences were deemed significant at $P \leq 0.05$. Male co-mingling body weight gain were subjected to a 2 x 2 factorial ANOVA (Diet and Co-mingling status) with main effects significantly different at $P \leq 0.05$.

RESULTS

Experiment 1

Over the course of the Experiment 1, no differences were observed with respect to body weights of male and female replacement broiler breeders of genetic Line A (Table 3.1). With respect to pen uniformity as measured by coefficient of variation, Line A males fed the integrator diet had improved ($P \leq 0.05$) uniformity on days 28 and 42 as evidenced by reduced pen coefficient of variation (Table 3.1). Dietary differences were not observed to impact flock uniformity in Line A females throughout the entire experiment. In Line B replacement broiler breeders, differences in body weight and coefficient of variation were influenced by diet. Line B females fed the breeder recommended diet had increased ($P \leq 0.05$) body weight on day 21 as compared to females fed the integrator diet. This observed difference in body weight persisted through the completion of the experiment on day 42 (Table 3.2). Line B females fed the breeder recommended diet also had improved ($P \leq 0.05$) pen uniformity as evidenced by a reduced coefficient of variation on day 28 and 42 compared to the integrator diet (Table 3.2). Line B males fed the breeder recommended diet were observed to have an increase in body weights ($P = 0.071$) of 50 g and reduced coefficient of variation ($P=0.073$) at the conclusion of the experiment, however neither one of these parameters were significant at 0.05.

Table 3.1 Body weights and flock uniformity of Line A replacement broiler breeders vaccinated with a live oocyst vaccine fed two different diets through 42 days of age reared on new litter

Diet	Age (d) 0	7	14	21	28	35	42
				Body Weight (g)			
Females							
Integrator	35.9 ± 0.01	95.6 ± 1.0	210.2 ± 1.5	396.6 ± 5.4	496.3 ± 5.4	477.7 ± 6.1	631.9 ± 10.2
Breeder Rec.	35.9 ± 0.01	94.1 ± 1.6	205.4 ± 2.9	389.7 ± 4.4	496.1 ± 3.2	480.0 ± 2.8	644.9 ± 3.3
Males							
Integrator	42.6 ± 0.1	135.1 ± 2.4	291.0 ± 3.8	562.8 ± 15.8	657.3 ± 11.4	722.5 ± 8.6	1030.4 ± 12.8
Breeder Rec.	42.4 ± 0.1	135.3 ± 3.1	285.9 ± 5.9	551.4 ± 15.3	653.1 ± 12.1	718.2 ± 10.4	1013.6 ± 9.0
				Flock Uniformity (Coefficient of Variation)			
Females							
Integrator	7.3 ± 0.01	19.5 ± 0.6	19.4 ± 0.9	18.9 ± 0.6	17.8 ± 0.8	17.1 ± 0.7	18.6 ± 0.7
Breeder Rec.	7.3 ± 0.02	19.2 ± 0.8	17.9 ± 0.8	18.2 ± 0.7	16.8 ± 0.6	16.7 ± 0.7	17.4 ± 0.8
Males							
Integrator	7.5 ± 0.2	16.9 ± 2.4	13.0 ± 1.0	14.4 ± 1.3	12.9 ± 0.8*	12.8 ± 0.8	13.2 ± 0.9*
Breeder Rec.	7.4 ± 0.2	16.1 ± 1.0	15.5 ± 1.0	15.9 ± 1.5	16.4 ± 1.3*	15.5 ± 1.4	16.1 ± 1.5*

* Indicates significant differences at $P \leq 0.05$ between diets within sex.

Table 3.2 Body weights and flock uniformity of Line B replacement broiler breeders vaccinated with a live oocyst vaccine fed two different diets through 42 days of age reared on new litter

Diet	Age (d) 0	7	14	21	28	35	42
				Body Weight (g)			
Females							
Integrator	37.3 ± 0.03	120.4 ± 2.1	267.2 ± 3.2	424.6 ± 3.3*	585.6 ± 4.0*	573.3 ± 3.8*	792.9 ± 9.6*
Breeder Rec.	37.3 ± 0.03	119.8 ± 3.3	278.6 ± 6.7	442.0 ± 6.0*	605.4 ± 5.3*	599.2 ± 6.3*	839.6 ± 9.1*
Males							
Integrator	37.5 ± 0.007	131.3 ± 2.4	299.1 ± 3.6	539.6 ± 10.7	725.3 ± 13.8	796.2 ± 12.4	1107.8 ± 16.7
Breeder Rec.	37.5 ± 0.01	134.1 ± 2.8	315.0 ± 5.7	557.8 ± 17.1	727.1 ± 11.6	819.4 ± 13.2	1157.0 ± 16.8
				Flock Uniformity (Coefficient of Variation)			
Females							
Integrator	6.6 ± 0.2	12.6 ± 1.2	13.3 ± 1.2	14.9 ± 1.2	15.0 ± 1.1*	13.9 ± 1.0	14.0 ± 0.8*
Breeder Rec.	6.5 ± 0.1	11.3 ± 0.4	11.9 ± 0.7	12.2 ± 0.7	11.8 ± 0.5*	11.7 ± 0.4	11.5 ± 0.6*
Males							
Integrator	5.7 ± 0.04	12.5 ± 0.8	14.0 ± 1.2	15.3 ± 1.2	15.3 ± 1.4	15.6 ± 1.7	17.0 ± 1.3
Breeder Rec.	5.6 ± 0.1	10.2 ± 1.0	11.5 ± 0.4	14.1 ± 0.8	13.2 ± 1.1	12.9 ± 1.1	13.9 ± 0.8

* Indicates significant differences at $P \leq 0.05$ between diets within sex

The impact of co-mingling on male body weight was uniform across both genetic lines. Male body weight gain was negatively ($P \leq 0.05$) affected in both genetic lines during the two seven day periods following the integration of the males into the female pens as compared to the males that were continued to be reared separately (Table 3.3). Line A co-mingled males were observed to actually lose weight during the first week of co-mingling. Body weight gain was increased ($P \leq 0.05$) in Line B males when fed the breeder recommend diet as compared to the integrator diet over the first seven days of co-mingling (Table 3.3). Female performance was unaffected due to male co-mingling (data not shown).

Experiment 2

Throughout the experiment, no significant differences were observed between dietary treatments in the Line A males or females (Table 3.4). Body weights of Line A females were similar between diets with only a seven gram difference at the conclusion of the trial. On day 42, Line A males fed the breeder recommended diet had an increased ($P=0.076$) body weight over the males fed the integrator diet but did not reach the level of significance at ($P \leq 0.05$). At the conclusion of the experiment, no differences were observed with respect to ending flock uniformity as measured by coefficient of variation in either gender of Line A replacement broiler breeders fed either dietary program. However, Line A female broiler breeders fed the integrator diet had improved ($P \leq 0.05$) pen uniformity on day 21 compared to the females fed the breeder recommended diet (Table 3.4).

Table 3.3 Body weight gain of replacement broiler breeder males of two genetic lines fed two different diets varying in protein level under two different rearing conditions (co-mingling with females versus separately reared) on new litter

	Rearing Status					
	Age (d)					
	28 to 35			35 to 42		
	Separate	Co-Mingled	Mean	Separate	Co-Mingled	Mean
Line A Males						
Diet						
Integrator	56.0 ± 3.8	-12.1 ± 10.9	22.0 ± 12.9	307.6 ±13.1	223.6 ± 7.9	265.6 ± 16.7
Breeder Rec.	60.1 ± 4.3	-1.7 ± 11.6	29.2 ± 12.1	295.4 ± 7.1	220.2 ± 11.9	257.8 ± 14.6
Mean	58.1 ± 7.6 [a]	-6.9 ± 2.8 [b]		301.5 ± 6.7[a]	221.9 ± 7.3[b]	
Line B Males						
Diet						
Integrator	67.5 ± 3.1	-8.1 ± 9.3	29.7 ± 13.9[b]	311.7 ± 5.9	240.2 ± 23.8	276.0 ± 16.2
Breeder Rec.	87.7 ± 4.6	52.6 ± 8.0	70.2 ± 14.5[a]	337.6 ± 8.3	231.2 ± 22.6	284.4 ± 21.3
Mean	77.6 ± 4.3[a]	22.3 ± 6.5[b]		324.7 ± 6.4[a]	235.7 ± 15.3[b]	

[a,b] Main effect means with different superscripts differ significantly at $P \leq 0.05$.

Table 3.4 Body weights and flock uniformity of Line A replacement broiler breeders vaccinated with a live oocyst vaccine fed two different diets through 42 days of age on used litter

Diet	Age (d) 0	7	14	21	28	35	42
				Body Weight (g)			
Females							
Integrator	43.0 ± 0.02	136.0 ± 1.5	280.8 ± 4.2	344.0 ± 3.3	406.0 ± 3.3	470.4 ± 5.2	562.9 ± 3.1
Breeder Rec.	42.9 ± 0.03	135.8 ± 0.8	282.1 ± 1.8	348.9 ± 1.6	408.9 ± 2.3	476.9 ± 3.6	569.1 ± 5.0
Males							
Integrator	46.0 ± 0.1	154.5 ± 2.5	312.7 ± 21.8	598.5 ± 42.5	650.4 ± 31.0	755.4 ± 29.4	926.5 ± 30.6
Breeder Rec.	46.1 ± 0.02	161.4 ± 1.7	347.9 ± 9.8	689.4 ± 21.3	704.5 ± 13.1	816.3 ± 8.6	990.0 ± 6.2
				Flock Uniformity (Coefficient of Variation)			
Females							
Integrator	6.2 ± 0.01	8.8 ± 0.5	9.5 ± 0.8	8.6 ± 0.7*	9.7 ± 0.7	10.5 ± 0.7	10.9 ± 0.7
Breeder Rec.	6.2 ± 0.02	9.6 ± 0.5	11.3 ± 0.4	10.8 ± 0.5*	10.8 ± 0.4	11.5 ± 0.5	11.9 ± 0.5
Males							
Integrator	6.4 ± 0.1	11.4 ± 0.8	11.5 ± 1.0	12.4 ± 1.2	12.8 ± 1.0	12.7 ± 0.8	12.5 ± 1.0
Breeder Rec.	6.4 ± 0.1	9.5 ± 0.6	10.8 ± 0.8	11.4 ± 0.9	12.2 ± 2.1	11.1 ± 0.9	11.7 ± 0.8

* Indicates significant differences at $P \leq 0.05$ between diets within sex.

Increases in average body weight ($P \leq 0.05$) of in Line B male and female replacement broilers breeders were observed when feeding the breeder recommended diet as opposed to the integrator diet. Both male and female body weights were immediately impacted with increases ($P \leq 0.05$) beginning on day 7 (Table 3.5). This trend of increased ($P \leq 0.05$) body weight in breeder recommended diet fed breeders was observed for both genders through the entire experiment. With respect to final flock uniformity of Line B replacement broiler breeders, no differences in pen coefficients of variation were observed between dietary programs regardless of gender.

Similar responses on body weight gain of male replacement broiler breeders due to co-mingling were observed as in Experiment 1 with co-mingling having a negative impact ($P \leq 0.05$) on the performance of both genetic lines. Co-mingling reduced body weight gain of male replacement broiler breeders in both genetic lines over the last 14 days of the experiment (Table 3.6). Line A males performed similar regardless of dietary program however, weight gain of Line B males was influenced due to dietary program. The Line B males fed the breeder diet were observed to have increased body weight gain for the two-seven day periods following the integration of males into the female pens (Table 3.6).

DISCUSSION

It is recognized that different planes of nutrition can influence the growth performance of replacement broiler breeders (Lilburn *et al*., 1989; Lilburn and Myers-Miller, 1990) which was supported by observations in this study. Varying dietary protein levels can impact early broiler breeder performance as increases in body weights were

Table 3.5 Body weights and flock uniformity of Line B replacement broiler breeders vaccinated with a live oocyst vaccine fed two different diets through 42 days of age on used litter

Diet	Age (d) 0	7	14	21	28	35	42
	Body Weight (g)						
Females							
Integrator	34.6 ± 0.01	101.2 ± 1.2*	209.4 ± 11.0*	309.2 ± 7.8*	419.8 ± 8.8*	515.1 ± 9.7*	635.1 ± 12.3*
Breeder Rec.	34.6 ± 0.01	109.6 ± 1.4*	255.4 ± 3.1 *	348.3 ± 2.6*	466.5 ± 5.1*	577.7 ± 6.0*	725.1 ± 8.4*
Males							
Integrator	33.3 ± 0.01	105.1 ± 2.0*	256.1 ± 6.4*	534.6 ± 13.5*	621.9 ± 10.5*	755.1 ± 7.7*	932.9 ± 11.0*
Breeder Rec.	33.3 ± 0.03	114.9 ± 4.2*	282.9 ± 10.5*	590.3 ± 10.8*	675.3 ± 9.2*	843.7 ± 13.7*	1047.2 ± 17.7*
	Flock Uniformity (Coefficient of Variation)						
Females							
Integrator	5.1 ± 0.01	12.2 ± 0.6	12.5 ± 0.5	11.5 ± 0.9	11.9 ± 0.8	12.8 ± 0.8	12.1 ± 0.8
Breeder Rec.	5.1 ± 0.01	12.4 ± 0.4	12.9 ± 0.6	10.6 ± 0.5	11.4 ± 0.5	12.0 ± 0.6	12.4 ± 0.5
Males							
Integrator	6.5 ± 0.1	12.3 ± 0.1	13.8 ± 1.2	14.2 ± 1.6	14.8 ± 1.4	13.4 ± 0.9	12.6 ± 0.8
Breeder Rec.	6.3 ± 0.1	12.8 ± 1.1	13.7 ± 1.7	13.2 ± 1.7	12.8 ± 1.7	12.6 ± 1.7	12.2 ± 1.5

* Indicates significant differences at $P \leq 0.05$ between diets within sex

Table 3.6 Body weight gain of replacement broiler breeder males of two genetic lines fed two different diets varying in protein level under two different rearing conditions (co-mingling with females versus separately reared) on used litter

	Rearing Status Age (d) 28 to 35			35 to 42		
	Separate	Co-Mingled	Mean	Separate	Co-Mingled	Mean
Line A Males						
Diet						
Integrator	105.4 ± 3.1	77.5 ± 3.9	91.5 ± 4.9	171.1 ± 8.2	104.2 ± 6.3	137.7 ± 10.7
Breeder Rec.	109.6 ± 5.6	84.7 ± 9.3	97.2 ± 3.4	173.8 ± 6.1	105.4 ± 11.7	139.6 ± 5.1
Mean	107.5 ± 3.1[a]	81.1 ± 4.9[b]		172.5 ± 4.8[a]	104.8 ± 6.2[b]	
Line B Males						
Diet						
Integrator	133.0 ± 10.8	79.7 ± 12.8	106.4 ± 10.3[b]	177.7 ± 6.1	115.9 ± 14.7	146.8 ± 6.4[b]
Breeder Rec.	169.9 ± 6.7	111.6 ± 6.5	140.8 ± 12.0[a]	203.4 ± 7.0	181.6 ± 21.2	192.5 ± 15.9[a]
Mean	151.5 ± 8.6[a]	95.7 ± 9.0[b]		190.6 ± 6.1[a]	148.8 ± 17.2[b]	

[a,b] Main effect means with different superscripts differ significantly at $P \leq 0.05$.

observed in both experiments. However, genetic background of the replacement breeders may determine if subtle dietary alternations will impact performance. A reduction in dietary protein level did not effect performance in Line A replacement broiler breeders while significant impacts were observed in Line B replacement broiler breeders with a similar reduction in protein concentration.

In both experiments, little effect was observed on performance parameters between the breeder recommended diet and the integrator diet in Line A males and females. However, average body weights of Line B replacement broiler breeders were significantly impacted by dietary program. Increased body weights were observed in males and females fed the breeder recommended diet when compared to the integrator program. Yassile and Liburn (1998) also observed differences in body weights due to dietary protein level in young broiler breeders with increased body weight when fed a diet consisting of 19% protein as compared to breeders fed a 15% protein diet. The data in the current two experiments agree with Yassile and Liburn (1998), while indicating that body weight of young replacement breeders can be impacted by dietary protein and amino acid profile, however the differences in protein concentration in the experiments where more subtle.

In both experiments, dietary formulation and gender was observed to impact pen uniformity as measured by coefficient of variation, however improvements were not always associated with feeding the breeder recommend diet, as was seen with body weight. In Experiment 1, flock uniformity of Line A males fed the integrator diet was improved on days 28 and 42, however with respect to Line A females, diet had no impact on flock uniformity throughout the experiment. Line B female flock uniformity was positively

influenced when fed the breeder recommended diet. Similar improvements ($P = 0.073$) were observed in Line B males fed the breeder diet, however these improvements did not reach the stated level of significance ($P \leq 0.05$). This indicates that genetic line, gender and diet can have a significant impact on the future performance of replacement broiler breeders. At the conclusion of Experiment 2, no differences were observed with respect to final flock uniformity in Line A male or female replacement broiler breeders fed either dietary program. This observation was different than in Experiment 1 with improvement in uniformity in the Line A males fed the integrator diet. Flock uniformity is a key parameter that directs the success of a flock at the onset and duration of production. A flock with a highly uniform body weight helps to harmonize the onset of sexual maturity, creates good laying peaks, and enables producers to meet the nutrient requirements of the flock more accurately. One level of management optimizes the reproduction effect of a large proportion of birds (Lopez et al., 1994). The results from the current study indicate that dietary protein level can influence flock uniformity among young broiler breeders.

When evaluating the effects of co-mingling on male performance, average body weight gain was negatively affected in both experiments regardless of genetic strain. However, dietary formulation can be used to overcome the growth depression associated with co-mingling. In both experiments, males fed the breeder recommended diet yielded increased body weight gain in Line B males. Body weight gain is important to maintain among males at early stages of growth. Commercially, the 7 to 15 week of age period has been reported to be the most critical for effective management of the flock because of its association with lasting improvement in reproductive performance (Bruggeman et al., 1999)

as underfeeding can hurt male fertility. However, caution must also be taken to ensure that males do not become too heavy. Males that are too heavy during rearing tend to become over fleshed, generally have reduced persistency of semen production, and have reduced fertility compared to birds fed to meet target body weight recommendations (Nir et al., 1975; McDaniel et al., 1981). The data from the current two experiments indicate that co-mingling of males during the early rearing period negatively affects body weight gain immediately following integration with females. However, the long lasting effects of this practice on final male body weight prior to sexual maturity are not known and should be investigated. In conclusion, male co-mingling negatively impacts male body weight gain and primary breeder dietary recommendations should be followed to prevent negative effects on early male and female average body weight and uniformity depending on genetic line.

CHAPTER IV

INFLUENCE OF DIET ON OOCYST OUTPUT AND INTESTINAL LESION DEVELOPMENT IN LIVE OOCYST VACCINATED REPLACEMENT BROILER BREEDERS

INTRODUCTION

Avian coccidiosis is responsible for significant economic losses in the poultry industry world-wide, and is caused by *Eimeria* spp. (Chapman, et al., 2002). *Eimeria* are intracellular protozoan parasites that invade the intestinal mucosa and induce a degree of epithelial cell damage and inflammation (Garcia-Neto et al., 2000). Coccidiosis results in reduced body weight, enteritis, and death (Idris et al., 1997). Vaccination is an effective means to prevent and/or reduce the adverse effects of specific diseases in poultry (Mathis and Broussard, 2006). In replacement broiler breeding flocks, the use of live oocyst vaccines to protect against coccidiosis has proven to be successful in reducing infection and with vaccination stimulating immunity (Danforth et al., 2002). The key to success is the equilibrium that is established between the birds' continuous excretion of oocysts onto the litter and the protective immunity thereby stimulated (Williams et al., 2000). Surveys of oocyst cycling patterns within a typical broiler grow out show that the peak of oocyst shedding can occur between three and four weeks of age (Mathis et al., 2003).

Dietary composition, with respect to protein concentration, has been shown to influence production parameters during avian coccidiosis (Garcia-Neto et al., 2000). Management guides provided by primary breeders include recommendations on dietary

composition, however, in an effort to reduce dietary cost; these recommendations are not always closely followed. Previous research has demonstrated increased broiler breeder pullet body weights resulting from increased dietary protein levels during coccidiosis vaccination (Yassile et al., 1999), however the range between the two protein levels evaluated in this study was quite large and may not be representative of typical replacement broiler breeder pullet diets. Also, the effect of varying protein levels on oocyst cycling and lesion development in vaccinated replacement broiler breeders was not assessed. Therefore, the current study was conducted to evaluate gross and microscopic intestinal lesion development and observe oocyst cycling patterns in replacement broiler breeders following coccidiosis vaccination when fed a diet which meets recommendations of the primary breeder or a diet that has been commonly fed by a commercial integrator.

MATERIALS AND METHODS

This study consisted of two consecutive experiments following the same experimental design. The first experiment was conducted on fresh pine shavings as litter material and the second was conducted on built up litter from the first experiment. Animal care was provided in accordance with the Texas A&M University Institutional Animal Care and Use Committee (IACUC).

Animals and Management Practices

A total of 480 broiler breeder males (240 Line A and 240 Line B) and 1152 broiler breeder females from two different genetic lines (576 Line A and 576 Line B) were placed in floor pens and fed either an integrator diet or genetic line specific breeder recommended diet. The males were housed in six replicate pens per group resulting in 24 total pens (12 per

genetic line) at a final placement density of 1.75 ft^2 per bird. Females were housed in eight replicate pens per group resulting in 32 total pens (16 per genetic line) at a final placement density of 1.5 ft^2 per bird. Females and males were given access to half of the pen through days 14 and 28, respectively, then full pen access was given for the remainder of the experiment in order to mimic half house brooding in a commercial setting.

Both males and females of each genetic line were subjected to the following lighting schedule in a dark out rearing house: 24 hours of light with an intensity of 30 lux (Day 0-3), 18 hours of light with an intensity of 30 lux (Day 4-7), 12 hours of light with an intensity of 15 lux (Day 8-21), 8 hours of light with an intensity of 15 lux (Day 22-42).

Dietary Program

Each genetic line was fed one of two diets (genetic recommended or integrator). The integrator diet was the same for both genetic lines and there was not a difference in dietary specifications based on sex. The only significant difference between the two breeder recommended diets and the integrator dietary program was protein concentration and amino acid profile with the integrator diet having a lower concentration. All chicks were vaccinated with a live oocyst vaccine (Coccivac®-D) at the hatchery of the primary breeder prior to delivery. Upon arrival, males and females of each genetic line were wing banded, weighed, and randomly assigned to treatment based on placement weight. Feeding programs and schedules were specific with regard to genetic line. All Line A replacement broiler breeders were switched from a starter to grower diet on day 21 which is in line with the breeder recommendations and integrator program. Breeder recommendations for Line B required that a starter diet be fed through six weeks of age, however, Line B breeders fed the

integrator program were switched from a starter to grower diet on day 26 to maintain the integrator management program. Feed restriction for both genetic lines began on day 21 (Experiment 1) or day 14 (Experiment 2) with daily allocations and skip-a-day feeding commencing on day 28 and persisting through the completion of the experiment on day 42.

Oocysts Cycling Patterns

Oocysts cycling patterns were determined by collecting fresh fecal material between feeders and drinkers on days 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 29, 31, 33, 35, 37, 39, 41. Fecal samples were thoroughly homogenized and diluted with water prior to applying 10 µl of fecal solution on to a McMaster's counting chamber for total oocyst determination. Oocyst output was calculated based on quadruplicate counts/pen/day using light microscopy.

Lesion Assessment

One bird per pen was removed on three different days throughout the experiment for the assessment of lesion development due to infection induced by vaccine strain oocysts. Lesion assessment was determined on days 18, 24, and 35 for Experiment 1 and days 16, 23, and 37 for Experiment 2. To assess intestinal lesion development, one bird was removed from each pen, killed, and the intestines removed for evaluation of gross lesions associated with vaccination (wherein 0 is normal and 1, 2, 3, or 4 indicate increasing severity of infection) (Johnson and Reid, 1970). Sampling sites included the ascending and descending loops of the duodenum, five inches on each side of Meckel's diverticulum, and both ceca. In Experiment 2, microscopic lesion development was also determined in addition to gross intestinal lesions for each intestinal section. Microscopic lesions were assessed by observing

intestinal parasitic stages after preparing slides of mucosal scraping for each area of the intestine.

Statistical Analysis

Lesion scores for males and females of each genetic line on each day of sampling were analyzed via a one-way ANOVA. Differences in lesion development due to diet within gender and genetic line were deemed significant at $P \leq 0.05$.

RESULTS

Lesion Development: Experiment 1

In general, lesion development in live oocyst vaccinated replacement broiler breeders reared on fresh pine shavings was more pronounced on day 17 as compared to day 24 and 35, corresponding to peak oocyst output. On day 17 of sampling, Line A females fed the integrator diet had higher lesion scores ($P \leq 0.05$) in the upper region compared to the breeder recommended diet (Table 4.1). Line A males fed the integrator diet had higher lesion scores ($P \leq 0.05$) in the upper region of the intestine compared to males fed the breeder recommended diet on day 17 (Table 4.1). There was no dietary influence ($P \geq 0.05$) on lesion development on day 24 and 35 in all sampled regions of female and male Line A replacement broiler breeders. In the Line B females, no effect on lesion development due to diet on day 17, 24, and 35 was observed (Table 4.2). On day 17, males fed the integrator diet had increased ($P \leq 0.05$) lesion development throughout the mid intestine compared to breeder recommended fed males (Table 4.2).

Table 4.1 Gross lesion scores of Line A replacement broiler breeders vaccinated with a live oocyst vaccine fed two different diets through 42 days of age reared on fresh litter

	Lesion Score								
Females	Day 17			Day 24			Day 35		
Diet	Upper	Mid	Lower	Upper	Mid	Lower	Upper	Mid	Lower
Integrator	1.6 ± 0.4*	0.5 ± 0.2	0.5 ± 0.4	0.3 ± 0.2	0.0 ± 0.0	0.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.1
Breeder Rec.	0.6 ± 0.3	0.4 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	0.0 ±0.0	0.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.1
Males									
Integrator	1.5 ± 0.4*	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.3
Breeder Rec.	0.5 ± 0.2	0.2 ± 0.0	0.3 ± 0.2	0.5 ± 0.2	0.0 ± 0.0	0.7 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.3

*Indicates a significant increase in lesion score within sex due to diet at $P \leq 0.05$.

Table 4.2 Gross lesion scores of Line B replacement broiler breeder females vaccinated with a live oocyst vaccine fed two different diets through 42 days of age reared on fresh litter

	Lesion Score								
Females	Day 17			Day 24			Day 35		
Diet	Upper	Mid	Lower	Upper	Mid	Lower	Upper	Mid	Lower
Integrator	1.1 ± 0.3	0.8 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.2
Breeder Rec.	0.8 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.2
Males									
Integrator	1.3 ± 0.2	1.2 ± 0.2*	0.5 ± 0.2	0.3 ± 0.2	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.2
Breeder Rec.	1.8 ± 0.7	0.2 ± 0.4*	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	1.2 ± 0.2

* Indicates a significant difference in lesion score within sex due to diet at $P \leq 0.05$

Lesion Development: Experiment 2

Throughout Experiment 2, there was no dietary influence observed with gross lesion development in Line A or B males or females. Gross lesion development tended to be more pronounced on the first sampling day as compared to subsequent sampling days similar to Experiment 1 however, the differences between sampling days were more subtle than observed in Experiment 1. This may be related to the differences in litter used during the experiments (new versus built up).

In Line A male and females, microscopic lesion development was increased ($P \leq 0.05$) in the integrator fed replacement broiler breeders in the upper portion of the small intestine on Day 37, however no other differences were observed in any other intestinal locations on any other sampling day (Table 4.3). No differences in microscopic lesion development were observed in any location on any sampling day in Line B male and female replacement broiler breeders fed the integrator or breeder recommended diets (Table 4.4).

Table 4.3 Gross and microscopic lesion scores of Line A replacement broiler breeder females vaccinated with a live oocyst vaccine fed two different diets through 42 days of age reared on built up litter

Gross Lesion Score

Females	Day 16			Day 23			Day 37		
Diet	Upper	Mid	Lower	Upper	Mid	Lower	Upper	Mid	Lower
Integrator	0.1 ± 0.1	0.6 ± 0.2	0.9 ± 0.1	0.5 ± 0.3	0.5 ± 0.2	0.8 ± 0.4	0.0 ± 0.0	0.1 ± 0.1	1.0 ± 0.2
Breeder Rec.	0.3 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.5 ± 0.3	0.5 ± 0.2	0.8 ± 0.2	0.0 ± 0.0	0.3 ± 0.2	1.1 ± 0.2
Males									
Integrator	0.2 ± 0.3	1.3 ± 0.5	0.2 ± 0.3	0.5 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.0 ± 0.0	0.5 ± 0.3	0.8 ± 0.5
Breeder Rec.	0.0 ± 0.0	1.3 ± 0.5	0.7 ± 0.2	0.7 ± 0.4	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.4

Microscopic Lesion Score

Females	Day 16			Day 23			Day 37		
Diet	Upper	Mid	Lower	Upper	Mid	Lower	Upper	Mid	Lower
Integrator	0.8 ± 0.5	0.9 ± 0.2	0.6 ± 0.2	1.4 ± 0.5	0.4 ± 0.2	0.1 ± 0.1	1.0 ± 0.0*	0.1 ± 0.1	1.0 ± 0.2
Breeder Rec.	0.9 ± 0.2	1.4 ± 0.4	0.8 ± 0.2	1.1 ± 0.4	0.8 ± 0.2	0.3 ± 0.2	0.6 ± 0.2	0.4 ± 0.2	1.3 ± 0.3
Males									
Integrator	0.2 ± 0.3	0.2 ± 0.3	0.0 ± 0.0	0.5 ± 0.3	0.0 ± 0.0	0.8 ± 0.8	1.1 ± 0.3*	0.9 ± 0.3	1.4 ± 0.3
Breeder Rec.	0.2 ± 0.2	0.7 ± 0.2	0.2 ± 0.2	0.7 ± 0.4	0.2 ± 0.2	0.0 ± 0.0	0.5 ± 0.2	0.4 ± 0.2	1.1 ± 0.8

* Indicates a significant difference in lesion score within sex due to diet at $P \leq 0.05$.

Table 4.4 Gross and microscopic lesion scores of Line B replacement broiler breeder females vaccinated with a live oocyst vaccine fed two different diets through 42 days of age reared on built up litter

Gross Lesion Score

Females	Day 16			Day 23			Day 37		
Diet	Upper	Mid	Lower	Upper	Mid	Lower	Upper	Mid	Lower
Integrator	0.9 ± 0.1	0.9 ± 0.2	0.6 ± 0.2	0.9 ± 0.3	0.8 ± 0.2	0.4 ± 0.2	0.0 ± 0.0	0.6 ± 0.2	1.4 ± 0.2
Breeder Rec.	0.9 ± 0.3	0.8 ± 0.3	0.6 ± 0.2	0.3 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.0 ± 0.0	0.3 ± 0.2	1.0 ± 0.2
Males									
Integrator	0.3 ± 0.2	1.7 ± 0.4	0.3 ± 0.2	0.5 ± 0.4	0.7 ± 0.2	0.5 ± 0.2	0.0 ± 0.0	0.1 ± 0.2	0.7 ± 0.2
Breeder Rec,	0.0 ± 0.0	2.2 ± 0.5	0.5 ± 0.2	0.8 ± 0.3	0.2± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2	0.8 ± 0.2

Microscopic Lesion Score

Females	Day 16			Day 23			Day 37		
Diet	Upper	Mid	Lower	Upper	Mid	Lower	Upper	Mid	Lower
Integrator	0.6 ± 0.2	1.3 ± 0.3	0.6 ± 0.2	1.6 ± 0.5	0.5 ± 0.2	0.6 ± 0.6	0.3 ± 0.2	0.3 ± 0.2	1.8 ± 0.5
Breeder Rec.	1.0 ± 0.3	1.1 ± 0.2	0.4 ± 0.2	0.9 ± 0.3	1.0 ± 0.3	0.5 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	1.3 ± 0.5
Males									
Integrator	0.2 ± 0.2	0.7 ± 0.4	0.3 ± 0.4	1.0 ± 0.6	1.2 ± 0.7	0.3 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.9 ± 0.0
Breeder Rec.	0.3 ± 0.2	0.7 ± 0.2	0.3 ± 0.2	1.2 ± 0.5	0.3 ± 0.2	0.5 ± 0.2	0.1 ± 0.2	0.2 ± 0.0	0.8 ± 0.2

Oocyst Output: Experiment 1

With regard to Line A, females fed the breeder recommended diet were observed to have a higher level of oocyst output on day 16 as compared to females fed the integrator diet (Figure 4.1). On subsequent sampling days, three peaks of lower magnitude were observed through day 33. Line A females fed the integrator diet were observed to have an identifiable peak on day 16 as well, however, the magnitude was lower than the peak observed in the breeder recommended fed females (Figure 4.1). Following day 16, several indistinct peaks of oocyst shedding were observed with the highest magnitude identified on days 35 and 41. Oocysts shedding patterns indicate a delay in peak shedding of vaccine strain *Eimeria* in the Line A females fed the integrator diet as compared to the females fed the breeder recommended diet. Line A males fed either the integrator diet or the breeder recommended diet were observed to have distinguishable peaks of oocyst output on days 16 and 26. Line A males fed the integrator diet were associated with a much higher magnitude of peak shedding on day 16 (Figure 4.2). Following day 16, oocysts shedding patterns were similar for males fed both the integrator and breeder recommended diets.

Line B females fed the breeder recommended diet had a single distinguishable peak of oocyst output on day 14 while low numbers of oocysts were observed throughout the remainder of the experiment following this peak. Females fed the integrator diet had identifiable peaks of shedding on days 16 and 37, with the greatest magnitude observed on day 16 (Figure 4.3). The magnitude of this peak was much higher as compared to the breeder recommended fed females (Figure 4.3). Line B males fed the breeder recommended diet were associated with a single identifiable peak of oocyst output on day 16 (Figure 4.4).

The magnitude of the single peak was higher than the observed peak for the integrator fed males during the same period. Males fed the integrator diet were observed to have two distinguishable peaks on days 18 and 24, with the highest magnitude observed on day 18 (Figure 4.4).

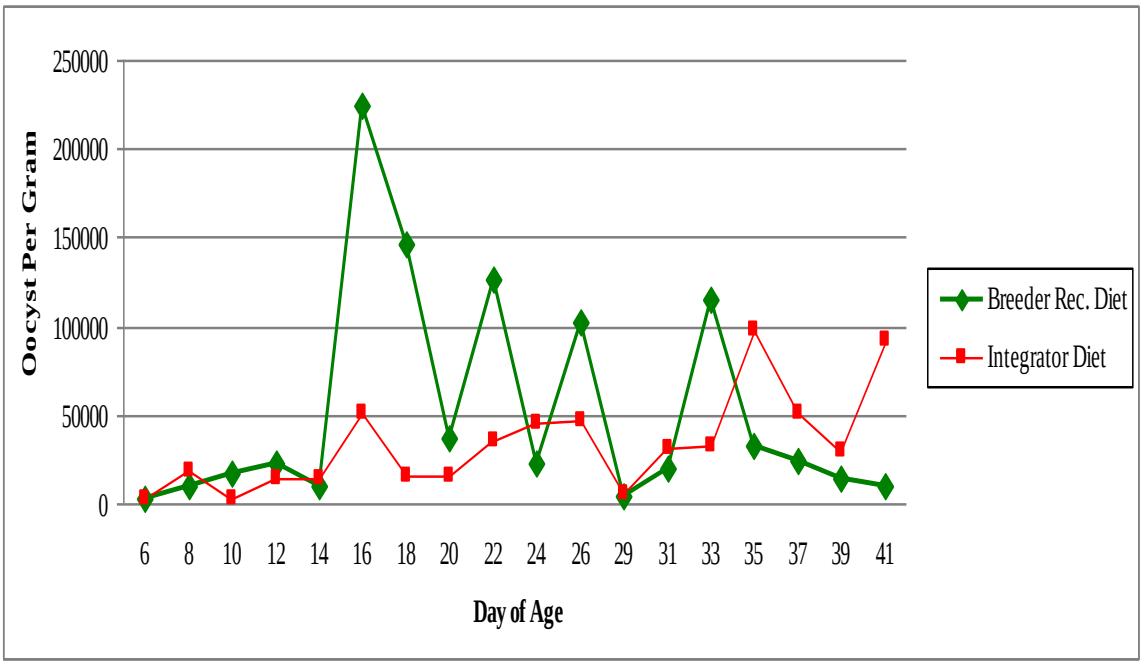

Figure 4.1 Oocyst cycling patterns of Line A replacement broiler breeder females vaccinated with a live oocyst vaccine fed two different diets reared on fresh pine shavings through 42 days of age.

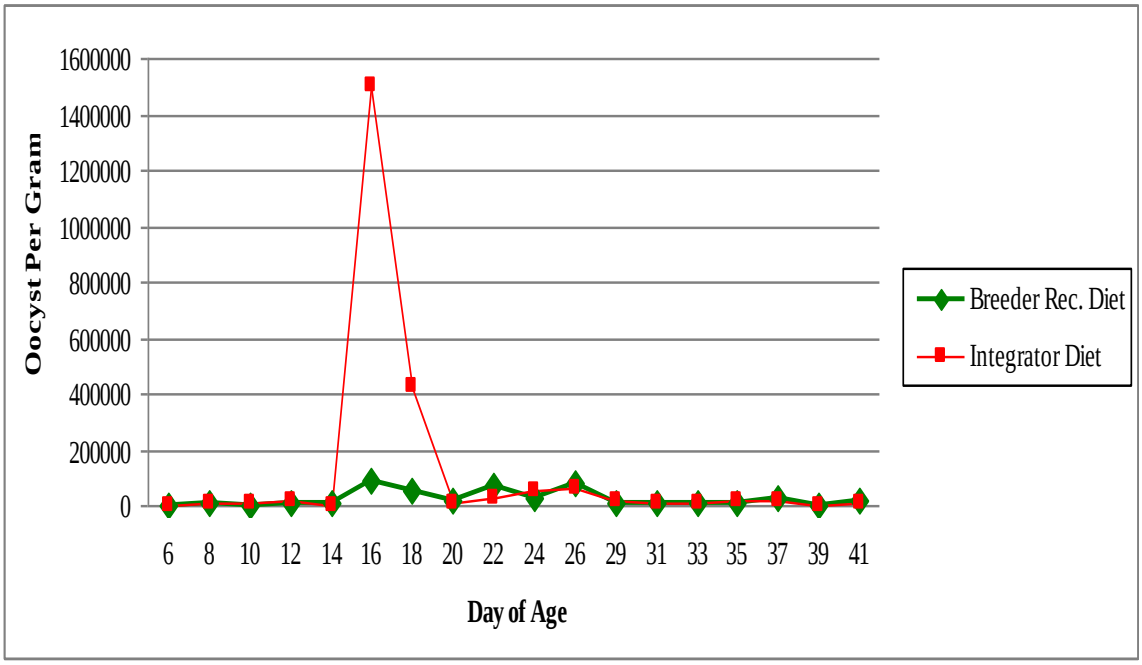

Figure 4.2 Oocyst cycling patterns of Line A replacement broiler breeder males vaccinated with a live oocyst vaccine fed two different diets reared on fresh pine shavings through 42 days of age.

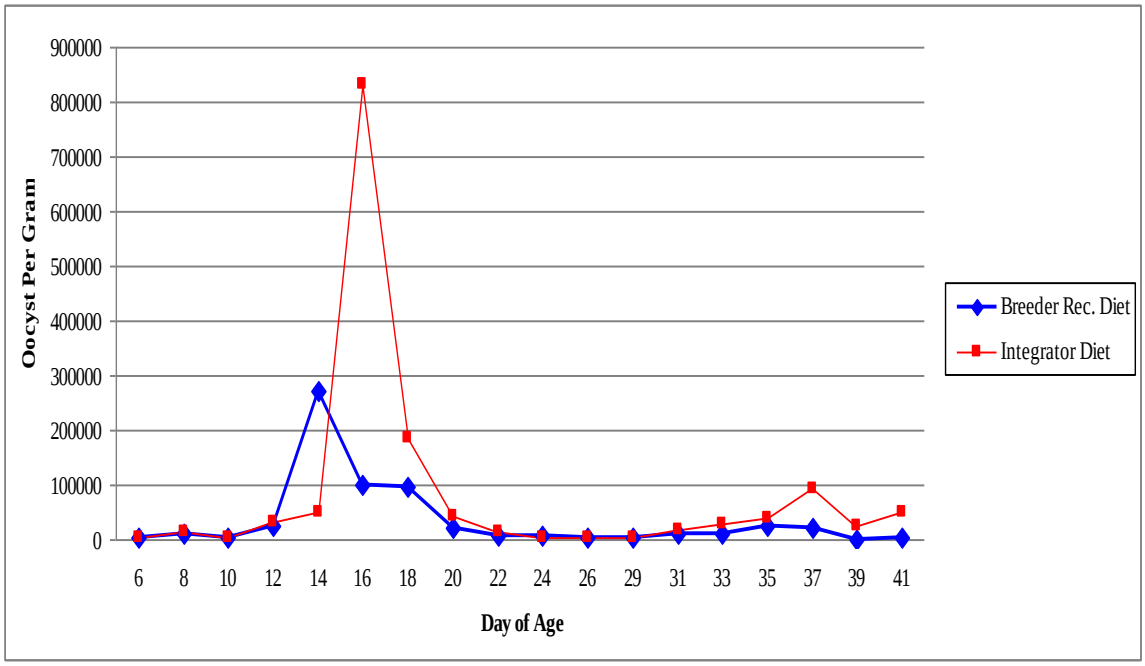

Figure 4.3 Oocyst cycling patterns of Line B replacement broiler breeder females vaccinated with a live oocyst vaccine fed two different diets reared on fresh pine shavings through 42 days of age.

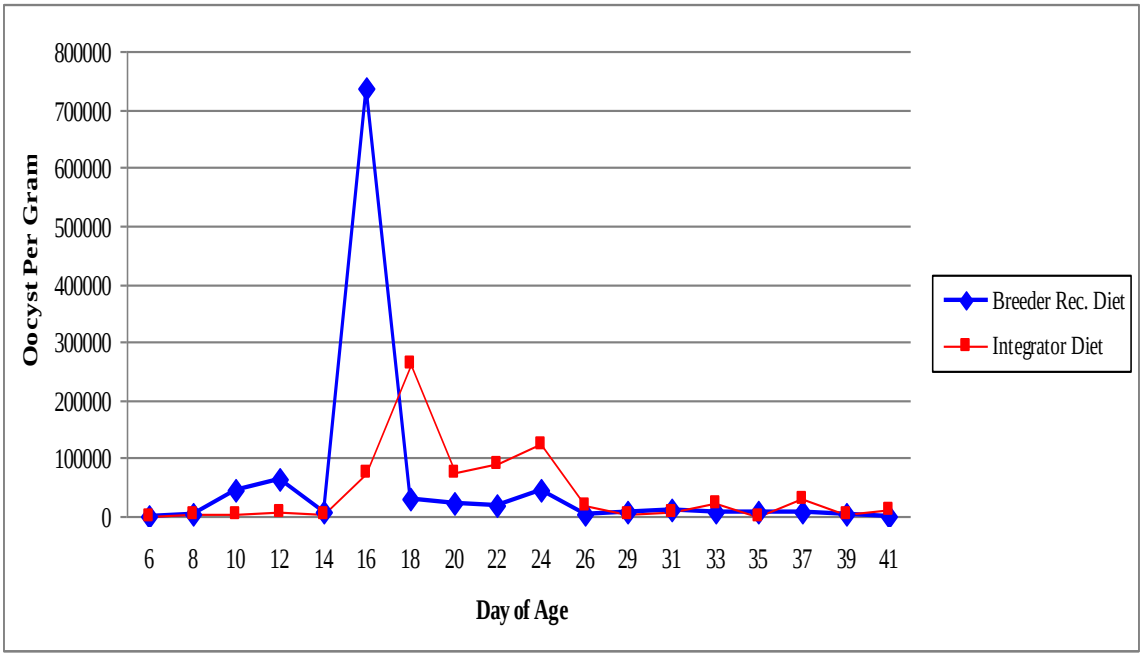

Figure 4.4 Oocyst cycling patterns of Line B replacement broiler breeder males vaccinated with a live oocyst vaccine fed two different diets reared on fresh pine shavings through 42 days of age.

Oocyst Output: Experiment 2

Line A females fed both, the breeder recommended diet and the integrator diet, were observed to have multiple peaks of oocyst output ranging from day 14 to day 35 (Figure 4.5). The highest magnitude of these peaks was observed on day 24 in both dietary treatments indicating a delay of peak shedding as compared to experiment 1. Line A males

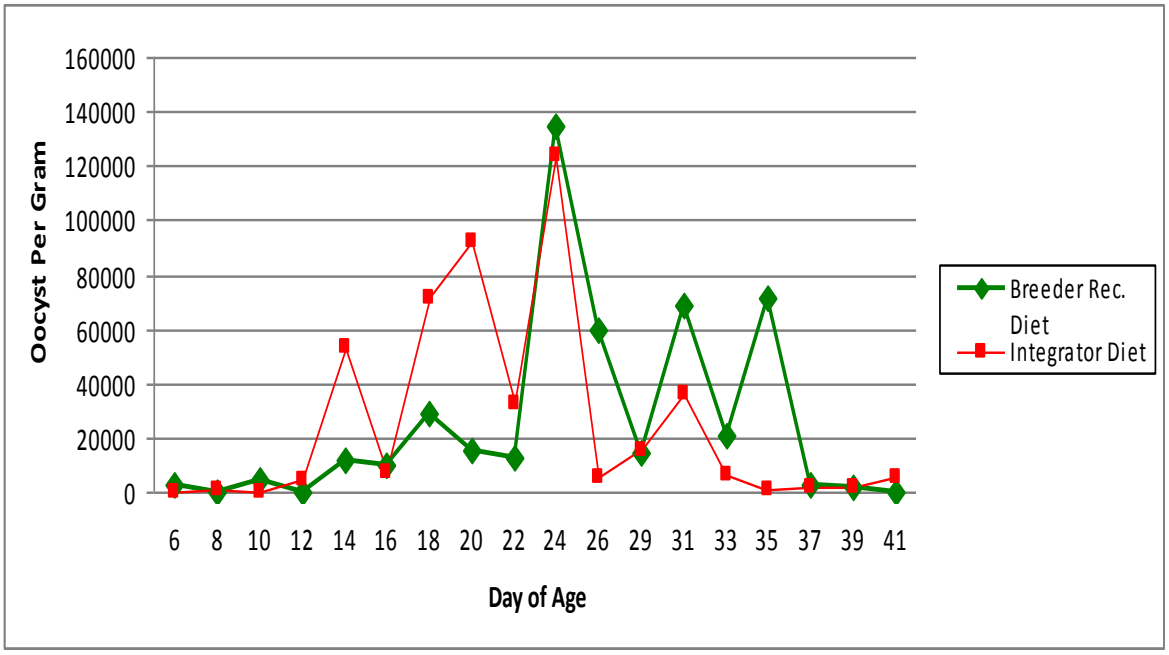

Figure 4.5 Oocyst cycling patterns of Line A replacement broiler breeder females vaccinated with a live oocyst vaccine fed two different diets reared on recycled pine shavings through 42 days of age.

fed the breeder recommended diet had two distinguishable peaks of oocyst output on days 29 and 35, with the greatest magnitude observed on day 35 (Figure 4.6). Males fed the integrator diet had identifiable peaks of shedding on days 26 and 33, with the greatest magnitude observed on day 33 (Figure 4.6). Similar to the Line A females, both dietary treatment groups of males were observed to have a delay in peak shedding as compared to experiment 1. The amount of oocysts shed during the peak period was less for males and females in experiment 2 as compared to experiment 1.

Line B females fed the breeder recommended diet had two distinguishable peaks of oocyst output on days 16 and 31, with the greatest magnitude observed on day 16 (Figure 4.7). Females fed the integrator diet had identifiable peaks of shedding on days 24 and 35, with the greatest magnitude observed on day 35 indicating a difference in shedding patterns between the two dietary treatments (Figure 4.7). Line B males fed the breeder recommended diet were associated with two identifiable peaks of oocyst output on days 18 and 29, with the greatest magnitude observed on day 29 (Figure 4.8). Males fed the integrator diet were observed to have three distinguishable peaks on days 16, 20, and 33, with the highest magnitude observed on day 33 (Figure 4.8).

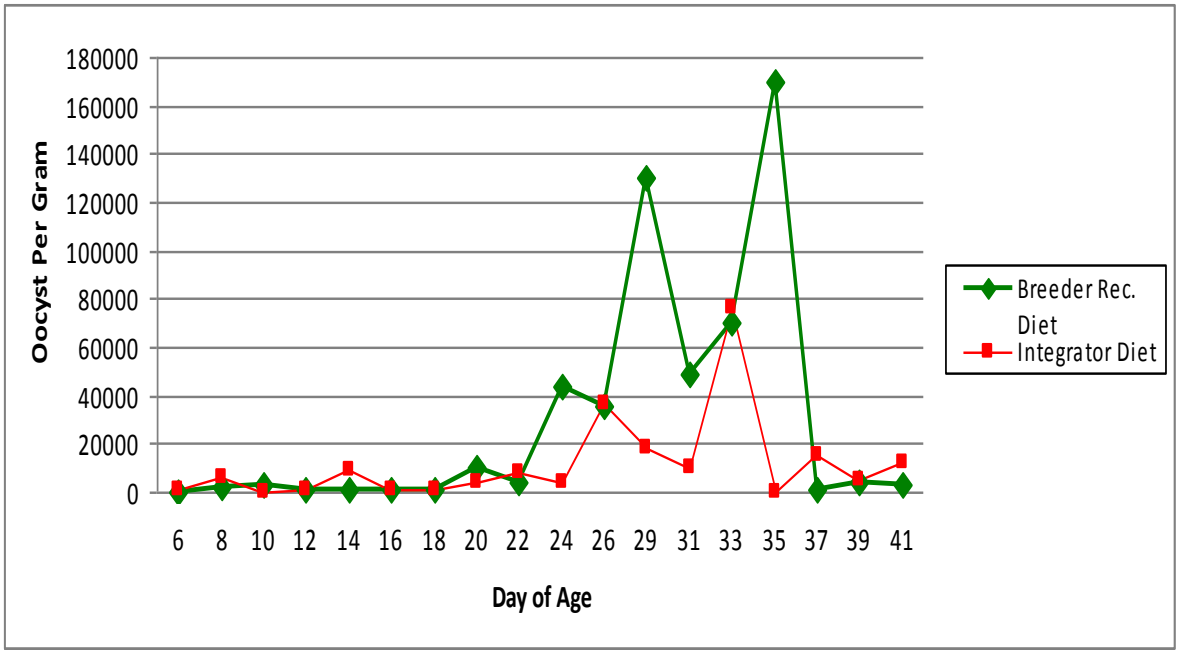

Figure 4.6 Oocyst cycling patterns of Line A replacement broiler breeder males vaccinated with a live oocyst vaccine fed two different diets reared on recycled pine shavings through 42 days of age.

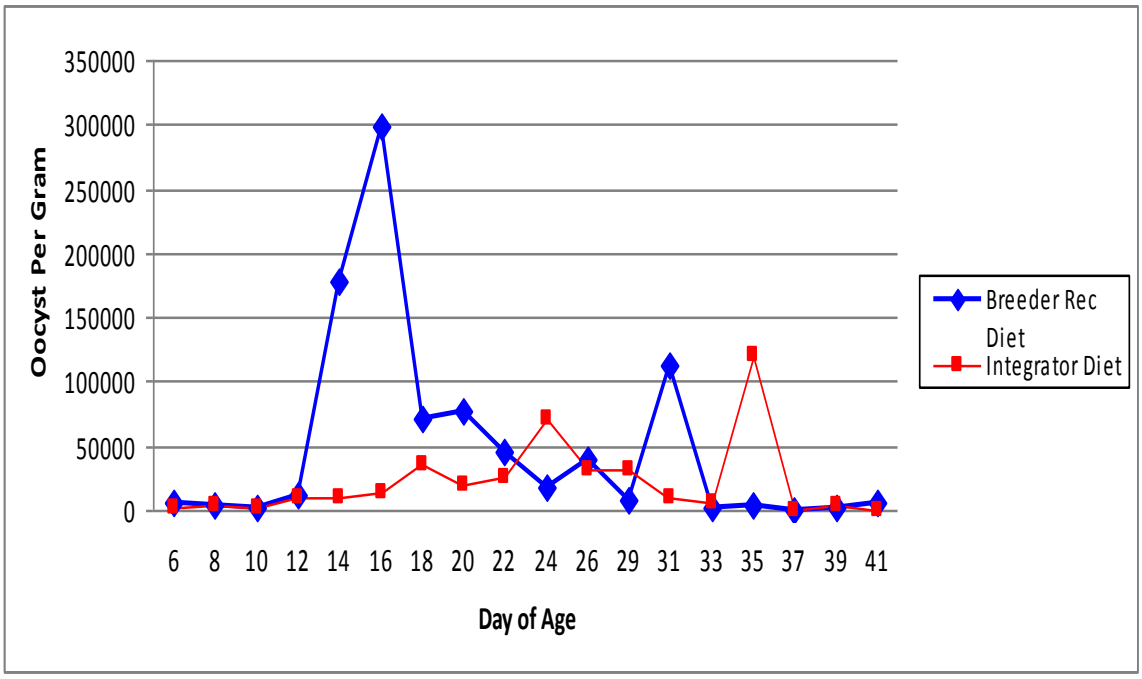

Figure 4.7 Oocyst cycling patterns of Line B replacement broiler breeder females vaccinated with a live oocyst vaccine fed two different diets reared on recycled pine shavings through 42 days of age.

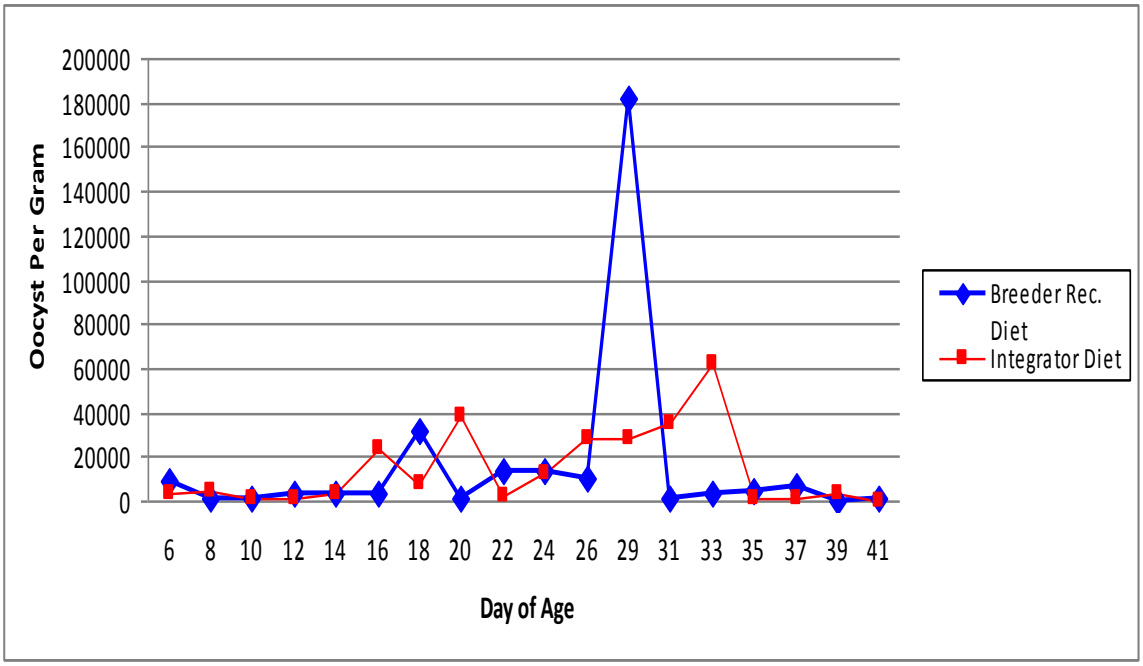

Figure 4.8 Oocyst cycling patterns of Line B replacement broiler breeder males vaccinated with a live oocyst vaccine fed two different diets reared on recycled pine shavings through 42 days of age.

DISCUSSION

From the epidemiological and diagnostic points of view, various parasitological factors may present specific characteristics when a live vaccine is used for mass control of coccidiosis in floor-reared poultry (Williams, 2003). When interpreting the efficacy and mode of action of live vaccines, it is necessary to understand the accumulation patterns of oocysts in the litter and the prevalence, timing and significance of gross lesions in the birds' intestines (Chapman et al., 2002; Williams and Andrews, 2001). The presence of numerous litter oocysts and the occurrence of visible coccidial lesions in intestinal tracts are expected

features of the use of live vaccines (Williams and Andrews, 2001). When rearing replacement broiler breeders, on fresh pine shavings, dietary composition impacts lesion development and oocyst shedding. In Experiment 1, lesions were more pronounced on day 17 of sampling among both genetic lines and gender, and dietary program influenced lesion development in males of both genetic lines. In Experiment 2, gross lesion development tended to be more pronounced on the first sampling day as compared to subsequent sampling days similar to Experiment 1, however the differences between sampling days were more subtle than observed in Experiment 1. Dietary program regardless of genetic line or gender had no impact on gross lesion development in Experiment 2. The change in this observation may possibly be related to the litter condition as Experiment 2 was conducted on built up litter from Experiment 1.

With respect to oocyst cycling patterns, initial peaks were observed between 14 to 16 days of age among males and females of both genetic lines when reared on fresh pine shavings. Following day 16, several distinct peaks of oocyst shedding were observed with the highest magnitude identified on days 35 and 41. This peak between 35 and 41 days of age may be related to caprophagy as skip a day feeding begin on day 28. In Experiment 2 when reared on built up litter, initial peaks of oocyst were delayed compared to the initial peaks observed in the first experiment. The highest magnitude of these peaks was observed on day 24 in both dietary treatments indicating a delay of peak shedding as compared to Experiment 1. This data correlates with the work conducted by Mathis et al., (2003), where peak oocyst shedding was observed between three and four weeks of age in broilers. The results of these experiments indicate that the magnitude of oocyst output and number of

identifiable peaks are influenced by genetic line and dietary composition. With respect to litter type the pattern and magnitude of oocyst output is altered. However, more research must be completed to verify this observation as breeder flock and time of year differed between these two experiments. The data shows a correlation between lesion development and oocyst output. On fresh shavings, lesions and oocyst output tend to be at their highest levels on day 17 which corresponds to the second cycle of vaccine derived oocysts. However, on built up litter, lesion incidence was similar on Day 17 and 23 correlating with the delay in peak oocyst shedding. In conclusion, lesion development and oocysts cycling patterns are directly related and are influenced by alterations of dietary protein level in live oocyst vaccinated replacement broilers breeders.

CHAPTER V

CONCLUSION

Currently, the US commercial poultry industry is using live oocyst vaccination as the main means for coccidiosis control and prevention in replacement broiler breeders. Maintaining early body weight of replacement broiler breeders is essential to ultimately yielding a productive flock. Dietary protein has been shown to positively influence performance in broilers and breeders when using live oocyst vaccination. Often breeder recommendations are not closely followed in the interest of cost savings and convenience. The objective of this research was to investigate the effect of dietary composition and co-mingling on male and female replacement broiler breeder performance, oocysts cycling, and lesion development in two commercially available genetic lines that were vaccinated with a live oocyst vaccine on day one of age.

The results of these two experiments indicate that dietary composition influences broiler breeder body weight, oocyst cycling, and lesion development in live oocysts vaccinated broiler breeders. Body weight was increased ($P \leq 0.05$) in both experiments when Line B breeders were fed the breeder recommended diet as compared to the integrator diet. Flock uniformity was improved ($P \leq 0.05$) with the breeder recommended diet in Line B breeders as compared to the integrator program. Co-mingling negatively influenced male body weight gain in both genetic lines, however, growth depression can be reduced in Line B males by feeding the breeder recommended diet as it increased ($P \leq 0.05$) body weight gain compared to the integrator diet. Genetic line appears to determine the ability of diet to

positively impact performance as Line A breeders were less sensitive to dietary alterations than Line B breeders.

Diet was also observed to impact lesion development in replacement broiler breeders as increased ($P \leq 0.05$) lesion development was observed in both genetic lines when fed the integrator diet as opposed to the specific primary breeder recommended diet. Litter condition alters the pattern and magnitude of oocyst output and lesion development. On fresh pine shavings, the highest incidence of gross lesions and the first identifiable peak, which tended to have the highest magnitude, was observed on day 16-18, whereas on built up litter, the peak of oocyst output is delayed and associated with similar incidence of lesions on days 17 and 23. The magnitude of oocyst output and number of identifiable peaks are influenced by genetic line and dietary composition.

These data indicate that co-mingling negatively impacts male body weight gain. Early flock performance, vaccine associated lesion development, and oocyst output are influenced by dietary composition depending on genetic line and gender. The results of these experiments confirm that subtle differences in dietary composition impact early replacement breeder performance and vaccine associated lesion development, and should be considered when rearing replacement broiler breeders.

REFERENCES

Adams, C., H.A. Vahl, A. Veldman. 1996. Interaction between nutrition and *Eimeria* acervulina infection in broiler chickens: development of an experimental infection model. British Journal of Nutrition. 75:867-873.

Allco, J.J., H. Profous-Juchelka, R.W. Meyes, B. Nare, D.M. Schmatz. 1999. Biosynthesis and metabolism of mannitol is developmentally regulated in the protozoan parasite *Eimeria tenella*. Journal of Parasitology 85:163-167.

Allen, P.C., H.D. Danforth, S.A. Gregory and P. Comens-Keller. 1997. Assessment of recombinant bovine somatotropin as an immunomodulator during avian coccidiosis: immunization with living oocysts. Poultry Science 76:1150-1155.

Allen, P.C., R.H. Fetterer. 2002. Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. Clinical Microbiology Reviews 15**:**58-65.

Augustine, P.C., H.D. Danforth, J.R. Barta . 1991. Development of protective immunity against *Eimeria tenella* and *E. acervulina* in White Leghorn chickens inoculated repeatedly with high doses of turkey coccidia. Avian Diseases 35:535-541.

Beach, J.R., J.C. Corl. 1925. Studies in the control of avian coccidiosis. Poultry Science 4: 83–93.

Bornstein, S., S. Hurwitz, Y. Lev. 1979. The amino acid and energy requirements of broiler breeder hens. Poultry Science 58:104-116.

Bowmaker, J.E., R.M. Gous. 1989. Quantification of reproductive changes and nutrient

requirements of broiler breeder pullets at sexual maturity. Br. Poult. Sci. 30:663-675.

Brake, J.J., D. Garlich, E.D. Peebles. 1985. Effect of protein and energy intake by broiler breeders during the pre-breeder transition period on subsequent reproductive performance. Poultry Science. 64:2335-2340.

Bruggeman, V., O. Onagbesan, E. D'Hondt, N. Buys, M. Safi, D. Vanmontfort, L. Berghmen, F. Vandesande, E. Decuypere. 1999. Effects of timing and duration of feed restriction during rearing on reproductive characteristics in broiler breeder females. Poultry Science 78:1424-1434.

Calnek, B. W. 1997. Diseases of Poultry. Wiley-Blackwell, Ames, IA.

Card, L. E., M.C. Nesheim. 1972. Diseases and parasites. Pages 244-273 in Poultry Production. 11th ed. Lea and Febiger, Philadelphia, PA.

Cave, N.A., 1984. Effect of a high-protein diet fed prior to the onset of lay on performance of broiler breeder pullets. Poultry Science. 63:1823-1827.

Chapman, H.D., T.E. Cherry, H.D. Danforth, G. Richard, M.W. Shirley, R.B. Williams. 2002. Sustainable coccidiosis control in poultry production: the role of live vaccines. International Journal of Parasitology. 32:617-629.

Chapman, H.D. 2009. A landmark contribution to poultry science--prophylactic control of coccidiosis in poultry. Poultry Science. 88:813-815.

Conway, D. P., M.E.McKenzie. 2007. Poultry Coccidiosis: Diagnostic and Testing Procedures. Blackwell Limited, Ames, IA.

Danforth H.D., P.C. Augustine, M.D. Ruff, R. McCandliss, R.L. Strausberg, M. Likel. 1989. Genetically engineered antigen confers partial protection against avian coccidial

parasites. Poultry Science 68:1643-1652.

Danforth, H D., T. E. Cherry, G. Richards, M.W. Shirley, R.B. Williams. 2002. Sustainable coccidiosis control in poultry production: the role of live vaccines. International Journal of Parasitology. 32:617-629.

Duffy, C.F., G.F. Mathis, R.F. Power. 2005. Effects of Natustat™ supplementation on performance, feed efficiency and intestinal lesion scores in broiler chickens challenged with *Eimeria* a*cervulina, Eimeria maxima and Eimeria tenella*. Veterinary Parasitology. 130:185-190.

Edgar, S. A. 1955. Sporulation of oocysts at specific temperatures and notes on the prepatent period of several species of avian coccidia. J. Parasitol. 41:214-216.

Fetterer, R. H., R. C. Barfield. 2003. Characterization of a developmentally regulated oocyst protein from *Eimeria tenella*. Journal of Parasitology 89:553-64.

Galmes, M.M., C.C. Norton, J. Catchpole. 1991. Comparison of resistance level and circulating IgG response in chickens experimentally inoculated with a multiple or single immunizing doses of *Eimeria acervulina*. Annals of Parasitology and Human Comparison. 66:144-148.

Garcia-Neto, M., G.M. Pesti, R.I. Bakalli. 2000. Influence of dietary protein level on the broiler chicken's response to methoinine and betaine supplements. Poultry Science. 79:1478-1484.

Graat, E., A.M. Henken, H.W. Ploeger, J. P., T.M. Noordhuizen, M.H. Vertommen. 1994. Rate and course of sporulation of oocysts of *Eimeria acervulina* under different environment conditions. Parasitology. 108:497-502.

Griffin, A. M., R. A. Renema, F.E. Robinson, M.J. Zuidhof. 2005 The influence of rearing light period and the use of broiler or broiler breeder diets on forty-two-day body weight, fleshing, and flock uniformity in broiler stocks. Journal of Applied Poultry Research 14:204-16.

Harms, R. H., C. F. Simpson, B. L. Damron, P.W. Waldroup. 1967. Influence of chronic intestinal coccidiosis on protein requirements of the laying hen. Poultry Science. 46:192-194.

Harms, R.H. 1992. An evaluation of the protein and lysine requirement for broiler breeder hens. Journal of Applied Poultry Research. 1:308-314.

Hein, H. 1976a. *Eimeria* acervulina, E. brunetti, and E. maxima: pathogenic effects of single or mixed infections with low doses of oocysts in chickens. Experimental Parasitology 39:415-421.

Hein, H.E. 1976b. *Eimeria acervulina, E. brunetti, E. maxima,* and *E. necatrix*: low doses of oocysts to immunize young chickens. Experimental Parasitology. 40:250-260.

Hudson, B. P., R. J. Lien, J.B. Hess. 2000. Effects of early protein intake on development and subsquent egg production of broiler breeder hens. Journal of Applied Poultry Research. 9:324-333.

Hudson, B. P., R. J. Lien, J. B. Hess. 2001. Effects of body weight uniformity and pre-peak feeding programs on broiler breeder hen performance. Journal of Applied Poultry Research. 10:24-32.

Idris, A. B., D.I. Bounous, M.A. Goodwin, J. Brown, E.A. Krushinskie. 1997. Lack of

correlation between microscopic lesion scores and gross lesion scores in commercially grown broilers examined for small intestinal *Eimeria* spp. Coccidiosis. Avian Diseases 41:388-391.

Joesph, N.S., F.E. Robinson, D.R. Korver, R.A. Renema. 2000. Effect of dietary protein intake during the pullet-to-breeder transition period on early egg weight and production in broiler breeders. Poultry. Science. 79:1790-1796.

Johnson, J. and M. Reid. 1970. Anticoccidial drugs: Lesion scoring techniques in battery and floor pen experiments with chickens. Experimental Parasitology. 28:30-36.

Joyner, L.P. 1982. Host and site specificity. Pages 36-57 in Biology of the Coccidia. University Park Press. Baltimore, MD.

Kheysin, Y. M., 1972. Chapter V. Sporulation of oocysts and their survival in the external environment. Pages 149–177 in: Life cycles of coccidia of domestic animals. K. S. Todd Jr., ed. University Park Press, London, UK

Leeson, S., J.D. Summers. 2000. Broiler-Breeder Production. Nottingham University Press. New York, NY.

Lilburn, M.S., K. Rilling, F. Mack, E.O. Mills, J.H. Smith. 1986. Growth and development of broiler breeders. Early plane of nutrition and growth rate. Poultry Science. 65:1070-1075.

Lilburn, M.S., K. Ngiam-Rilling, J.H. Smith. 1987. Relationships between dietary protein, dietary energy, rearing environment and nutrient utilization by broiler breeder pullets. Poultry Science. 66:1111-1118

Lilburn, M. S., K. Ngiam-Rilling, D. J. Myers-Miller. 1989. Growth and development of

broiler breeders. Independent effects of dietary formulation versus body weight on skeletal and muscle growth. Poultry Science. 68:1274-1281.

Lilburn, M. S., D. J. Myers-Miller. 1990. Effect of body weight, feed allowance, and dietary protein intake during the pre-breeder period on early reproductive performance of broiler breeder hens. Poultry Science. 69:1118-1125.

Lillehoj, E.P., H. Cheol, Y. Lillehoj, H.S. Lillehoj. 2000. Vaccines against the avian enteropathogens *Eimeria, Cryptosporidium and Salmonella.* Animal Health Research Reviews 1:47-65.

Long, P.L. 1973. Pathology and pathogenicity of coccidial infections. Pages 253-294 in: The Coccidia: *Eimeria,* Isospora, Toxoplasma, and Related Genera, University Park Press, Baltimore, MD.

Long, P.L., T.K. Jeffers. 1982. Studies on the stage of action of ionophorous antibiotics against *Eimeria*. Journal of Parasitology. 68:363-371.

Lopez, G., S. Lesson. 1994. Nutrition and broiler breeder performance: a review with emphasis on response to diet protein. Journal of Applied Poultry Research 3:303-11.

Lopez, G., S. Leeson. 1995. Response of broiler breeders to low-protein diets. Poultry Science. 74:685-695.

Mathis, G.F., R. Froyman, T. Irion, T. Kennedy. 2003. Coccidiosis control with toltrazuril in conjunction with anticoccidial medicated or nonmedicated feed. Avian Diseases. 47:463-469.

Mathis, G.F., C. Broussard. 2006. Increased level of *Eimeria* sensitivity to diclazuril after using a live coccidial vaccine. Avian Diseases. 50:321-324.

McDaniel, G.R., J. Brake, R.D. Bushong. 1981. Factors affecting broiler breeder performance. Relationship of daily feed intake to reproductive performance of pullet. Poultry Science. 60:307-312.

McDougald, L. R., A. L. Fuller, J. Solis. 1986. Drug sensitivity of 99 isolates of coccidia from broiler farms. Avian Diseases. 30:690-94.

Milkaski, W.P., J.K. Crooks, J. Prouse. 1994. Purification and characterization of serine-type proteases from *Eimeria tenella* oocysts. Journal of Parasitology. 24:189-195.

Morris, G.M., R.B. Gasser. 2006. Biotechnological advances in the diagnosis of avian coccidiosis and the analysis of genetic variation in *Eimeria*. Biotechnology Advances. 24:590-603.

Nakai, Y., T. Uchida, K. Kanazawa. 1992. Immunization of young chickens by trickle infection with *Eimeria tenella*. Avian Diseases. 36:1034-1036.

Nir, I., G.M.H. Waites, F.J. Cunningham. 1975. Obesity induced by force-feeding and accompanying changes in body temperature and fertility in male domestic fowl. British Poultry Science. 16:505-515.

Pearson, R.A., K.M. Herron. 1981. Effects of energy and protein allowances during lay on the reproductive performance of broiler breeder hens. Britain Journal of Poultry. Science. 22:227-239.

Pearson, R.A., K.M. Herron. 1982. Relationship between energy and protein intakes and laying characteristics in individually caged broiler breeder hens. Britain Journal of Poultry Science. 23:145-159.

Peek, H.W., J. M. Landman. 2006. Higher incidence of *Eimeria* spp. field isolates

sensitive for diclazuril and monensin associated with the use of live coccidiosis vaccination with Paracox -5 in broiler farms. Avian Diseases. 50:434-439.

Pellérdy, L.P. 1974. Coccidia and Coccidiosis. Berlin: Parey.

Proudfoot, F.G. 1980. The effects of dietary protein levels, ahermeral light and dark cycles, and intermittent photo periods on the performance of chicken broiler parent genotypes. Poult. Science. 59:1258-1267.

Schjeide, D.A., M. Wilkens, R.G. Candless, R.J. Munn, M. Peterson, E. Carlsen. 1963. Liver synthesis, plasma transport and structural alterations accompanying passages of yolk protein. Am. Zool. 3:167-184.

Schmatz, D.M. 1977. The mannitol cycle in *Eimeria*. Parasitology. 114:S81-S89

Sharma, V.D., M.A. Fernando. 1975. Effect of *Eimeria acervulina* infection on nutrient retention with special reference to fat malabsorption in chickens. Can. J. Comp. Med. 39:146-154.

Shirley, M.W., P. Bedrnik. 1997. Live attenuated vaccines against avian coccidiosis: success with precocious and egg-adapted lines of *Eimeria*. Parasitol. Today. 13:481-484.

Shirley, M.W., A.L. Smith, D.P. Blake. 2007. Challenges in the successful control of the avian coccidia. Vaccine. 25:5540-5547.

Siegel, P.B. E.A. Dunnington. 1985. Reproductive complications associated with selection for broiler growth. Pages 59-72 in Poultry Genetics and Breeding. W.G. Hill, J.M. Manson, and D. Hewitt, ed. British Poultry Science, Ltd., Harlow, UK.

Spratt, R.S., S. Leeson. 1987. Broiler breeder performance in response to diet protein and energy. Poultry Science. 66:683-693.

Vermeulen, A. N., D. C. Schaap, T.P. Schetters. 2001. Control of coccidiosis in chickens by vaccination. Veterinary Parasitology 100:13–20.

Waldenstedt, L., K. Elwinger, A. Lunde´n, P. Thebo, A. Uggla. 2001. Sporulation of *Eimeria maxima* oocysts in litter with different moisture contents. Poultry Science. 80:1412-1415.

Waldroup, P.W., K.R. Hazen, W.D. Bussell, Z.B. Johnson. 1976. Studies on the daily protein and amino acid needs of broiler breeder hens. Poultry Science. 55: 2342-2347.

Walsh, T. J., J. Brake. 1997. The effect of nutrient intake during rearing of broiler breeder females on subsequent fertility. Poultry Sci. 76:297–305.

Watkins, K.L., M.A. Brooks, T.K. Jeffers, P.V. Phelps, C.A. Ricks. 1995. The effect of *in ovo* oocyst or sporocyst inoculation on response to subsequent coccidial challenge. Poultry Science. 74:1597-1602.

Welch, C. C., C. M. Parsons, D.H. Baker. 1986. Further investigations of the dietary protein and Monensin level interrelationship in broiler chicks: influence of *Eimeria acervulina* infection, increased dietary protein, and level of feed intake. Poultry Science. 65:1939-1944.

Williams, R B., J. D. Johnson, S. J. Andrews. 2000. Anticoccidial vaccination of broiler chickens in various management programs: relationship between oocyst accumulation in litter and the development of protective immunity. Vet. Res. Commun. 24:309-325.

Williams, R.B., S.J. Andrews. 2001. The origins and biological significance of the

coccidial lesions that occur in chickens vaccinated with a live attenuated anticoccidial vaccine Avian Pathology. 30:215-220.

Williams, R. B. 2002. Historical article: fifty years of anticoccidial vaccines for poultry (1952-2002). Avian Diseases. 46:775-802.

Williams, R.B. 2003. Anticoccidial vaccination: the absence or reduction of numbers of endogenous parasites from gross lesions in immune chickens after virulent coccidial challenge Avian Pathology. 32: 535-543.

Wilson, H.R., R.H. Harms. 1984. Evaluation of nutrient specifications for broiler breeders. Poultry Science. 63: 1400-1406.

Yassile, J., M.S. Lilburn. 1998. Effects of dietary protein and strain on the growth of broiler breeders pullets from zero to five weeks of age. Poultry Science. 77: 1613-1619.

Yassile, J E., T. Morishita, M. Lilburn. 1999. Effects of dietary protein on restrict-fed broiler breeder pullets during a coccidial challenge. Poultry Science 78:1385-1390.

Yu, J.Y., R.R. Marquardt. 1974. Hyperplasia and hypertrophy of the chicken (*Gallus domesticus)* oviduct during a reproductive cycle. Poultry Science. 53:1096-1105.

Yun, C. H., H. S. Lillehoj, and K. D. Choi. 2000. *Eimeria tenella* infection induces interferon-γ production and intestinal lymphocyte subpopulation changes. Infection and Immunity. 68:1282–1288.

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